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The views expressed in this Bulletin by authors are not necessarily those of the Society or its Editorial Board.

NOTES TO AUTHORS

In addition to publishing papers (or abstracts or titles thereof) read at the Annual Congress of the Society, the *Bulletin* will accept for publication *original contributions*, subject to the following conditions:

1. Papers not directly related to Australia, New Zealand and New Guinea may not be accepted.

2. Taxonomic papers which describe new species will not be accepted unless any "type" material has been deposited at an Australian museum and evidence is presented of its Registration.

3. Manuscripts are to be typed, double-spaced and submitted in duplicate.

4. Photographs will be accepted only in exceptional circumstances.

5. Line drawings, graphs, etc., must be kept to a minimum.

6. All papers will be subject to refereeing.

7. Abbreviated titles of hournals cited in *"References"* must conform with the *World List of Scientific Periodicals* abbreviations.

8. 25 reprints will be made available to the author free; additional reprints will be provided at cost.

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ELEVENTH ANNUAL CONGRESS

THE FOLLOWING ARE PAPERS, ABSTRACTS AND TITLES OF PAPERS PRESENTED AT THE ELEVENTH ANNUAL CONGRESS OF THE AUSTRALIAN SOCIETY FOR LIMNOLOGY, HELD AT NARRANDERA, NEW SOUTH WALES, IN MAY, 1972.

INLAND AQUACULTURE AND LIMNOLOGY

(abstract only)

by

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Aquaculture in its broadest sense could be defined as "the management of aquatic stocks for individual or collective benefit." In most cases, the incentive behind aquaculture projects is commercial, and many such projects have been developed to a state of profitability. However there are other incentives, such as conservation, aesthetic and recreation demands, which must be considered.

In inland areas most of the water available for aquaculture can be regarded as fresh, and is found close to the coastline. In general, areas of high population density coincide with areas having useful freshwater supplies. As population increases, the various demands on localised freshwater resources must increase accordingly. It is already obvious that these demands, whether they be for domestic, agricultural, or industrial uses, are in conflict with conservation needs in many areas.

It will be in this climate of increasing demand conflict for a limited resource, i.e. water, that inland aquaculture as a concept will have to develop.

At present, commercial aquaculture is a minor occupation in inland areas, represented mainly by trout farms. However interest is growing in the commercial possibilities of inland aquaculture, particularly in relation to fish and crustaceans.

Non-commercial aquaculture projects, based on the various needs mentioned earlier, are concepts for the near future rather than the present, although State Fish Hatcheries are a current example. The need for this type of activity, in one form or another, must increase with population growth, and may outstrip the capabilities of Government-sponsored programmes. Commercial fish-out areas are of relevance in this context and provide a service outside the current sphere of Government activity.

As the commercial possibilities of inland aquaculture are explored and developed, the demand for suitable available resources must increase. This demand will be directed toward water and growing space. If growing space is available on freehold land, the resultant demand is for water only. However land is expensive, and it is reasonable to assume that demand will initially be directed toward the non-utilized coincident resources – water and space together – which may be available for aquaculture. This combination can only be found in those inland waters, both lentic and lotic, under the Crown's control. There are obvious mechanical problems in utilization of lotic habitat, and lentic situations are therefore preferred for aquaculture. It follows that inland lakes, whether natural or artificial, must be regarded as attractive propositions for potential commercial aquaculture.

In Victoria at least, the first overtures have already been performed, as specific applications for culture rights to inland lakes have been received. If these applications are successful, those in authority should prepare for a continued and aggressive demand for similar concessions.

Similar demands will develop elsewhere, for an increasing variety of habitats and species, and initial policy decisions made by Government will have far-reaching implications. For example, low-lying swampland close to permanent rivers can be converted quickly and cheaply to suitable aquaculture habitat. Northern areas, with elevated water temperatures, will also be recognised as potentially valuable culture sites, due to the possibility of increased growth rates and the resultant higher production.

The future of high-intensity aquaculture based on large water flows is questionable. Australia simply does not have an abundance of regular, flowing water that can be easily diverted to or through suitable culture sites. Gravity or artesian supply is essential for this type of operation, and suitable sites with the required quantity and quality of water are not common, especially in close proximity to major market outlets.

The first choice of commercial aquaculturers must therefore be those areas in which limnological disciplines find a great deal of their study material, interest, and concern for the future. Limnologists should no doubt be eager to comment on proposals for commercial utilization of inland aquatic habitats, but opportunity to influence future developments in this field will not be available indefinitely.

CHILODONELLA CYPRINI (MOROFF), A PARASITE OF FRESHWATER FISH AND ITS TREATMENT

by

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Chilodonella cyprini (Moroff) is a protozoan parasite of freshwater fishes and has been reported from most continents. In Europe it is a problem for both trout (*Salmo* spp.) and carp *(Cyprinus carpio)* hatcheries (Schäperclaus, 1935, Bauer and Nikol'skaya, 1957), and in the Middle East it causes heavy mortalities in warm water fish ponds (Sarig, 1968, 1971). The species also occurs in the cold and warm-water fisheries of the Sino-Indian region (Bauer, 1959). In North America, *C. cyprini* rarely causes trouble in salmonid hatcheries, but it has been encountered on warm-water fish, mainly during the winter and spring (Meyer, 1970).

Field samples taken in Victoria during the last 10 years have shown that *C. cyprini* occurs on a variety of native and introduced fish (Table 1). It has also been detected at the Snobs Creek Freshwater Fisheries Research Station and Hatchery on brown trout *(Salmo trutta)* fry (Woodland, 1960), where it causes considerable losses each year (Ashburner and Ehl – unpublished data).

At Snobs Creek *Chilodonella* has most frequently been found gliding over the gill filaments of the brown trout. It causes little or no damage to adults, but in fry (20-40 mm) heavy mortalities associated with gill hyperplasia can result from as few as two or three *Chilodonella* per gill filament depending on the associated environmental conditions.

In cold-water hatcheries a number of treatments have been used, including formalin, pyridylmercuric acetate (P.M.A.), copper sulphate, "Roccal" (alkyl benzalkonium chloride), sodium chloride, malachite green and methylene blue.

At Snobs Creek the first three treatments have been used satisfactorily a number of times, but they are costly, time consuming and toxic. The remaining treatments have been used under varying conditions, generally with success.

Two treatments have proved to be the most satisfactory. The first treatment involves keeping the fry in a two per cent solution of commercial sodium chloride in the hatching boxes for 20-30 minutes and disposing of any dead fish after the treatment. Although this treatment uses quite large quantities of salt, it is cheap, simple to administer, has a large safety factor, and is very successful, particularly if the source of re-infestation can be located.

The other treatment used for larger units (ponds and raceways), and also for larger fish, is a flush treatment with malachite green (zinc free). Malachite green is dripped into the unit at a rate which will produce a 0.2 mg per litre solution in a period of two hours. This treatment is repeated three days later if necessary, and under normal circumstances is used only during late autumn, winter, and early spring, as it appears to have a very low safety factor when used during the summer months.

TABLE 1

Fish sampled in Victoria on which *Chilodonella cyprini* has been detected (nomenclature of Lake 1971).

Family	Genus and Species	Common Name
Salmonidae	*Salmo gairdneri**	Rainbow trout
	*S. trutta**	Brown trout
Retropinnidae	*Retropinna semoni*	Australian smelt
Galaxiidae	*Galaxias sp.*	Galaxias
Cyprinidae	*Carassius auratus**	Goldfish
	*C. carassius**	Crucian carp
Serranidae	*Macquaria australasica*	Macquarie perch
	Maccullochella macquariensis	Murray cod
	M. mitchelli	Trout cod
Kuhliidae	*Nannoperca australis*	Southern pygmy perch
Gadopsidae	*Gadopsis marmoratus*	River blackfish
Gobiidae	*Morgurnda australis*	Striped gudgeon

*Species introduced to Australia.

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PRESUMPTIVE COLUMNARIS DISEASE AT SNOBS CREEK HATCHERY 1968

by

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Summary

An outbreak of presumptive Columnaris disease among stressed hatchery trout is recorded. Provisional diagnosis of the disease and successful treatment are described.

Columnaris disease is a common and widespread bacterial disease of freshwater fish. It is caused by the myxobacteria, *Chondrococcus columnaris* or *Cytophaga columnaris* (Bullock & McLaughlin, 1970) and has been found infecting tropical fish, warm-water fish and cold-water fish, under both artificial conditions of aquaria and fish hatcheries and naturally in rivers or lakes. The general symptoms and the bacteria responsible have been described by a number of authors (Anderson & Conroy, 1969, Amend, 1970, Pacha and Ordal, 1971).

The Snobs Creek Freshwater Fisheries Research Station and Hatchery carries rainbow trout (*Salmo gairdneri* Richardson) and brown trout (*S. trutta* L.) for stocking public waters. During the warmer months of the year, December to April, there is generally a minimal water supply to the hatchery. In March 1968, because of severe drought conditions, the normal water supply had to be supplemented by pumping 1/3 to 1/2 of the required water from the Goulburn River, whose temperature was substantially below that of the normal water supply. Due to an accident, this supplementary water was unavailable for 12 to 15 hours and during this period the fish in the raceway and pond units received only an intermittent water supply.

The following day the fish appeared to exhibit abnormal behaviour and were not feeding. Two days later the mortalities were abnormally high and the fish were found to have a severe infestation of *Trichodina* sp. and a milder infestation of *Chilodonella cyprini.* An apparent secondary fungal infection of the gills was also observed, though later this dark mass was found to consist of necrotic tissue and bacteria.

Gill smears showed large masses of gram negative thin rods. Before staining, these bacteria exhibited a slow sinuous movement and tended to form into columns or dome-shaped structures.

Cultures were attempted using material from the gills and plated onto Brain Heart Infusion Agar, Tryptone Soya Agar, Dextrose Sabouraud Agar, and Ordal's Agar (Bullock, 1961) and incubated at 20°C. for 48-72 hours.

The bacteria grew poorly on all media, though Tryptone Soya Agar gave the best growth. Ordal's Agar, a myxobacterial medium, which is slightly less solid than the other media, showed the characteristic patterns of the Columnaris disease organism (Davis, 1953). Other tests to confirm the diagnosis could not be carried out due to the poor growth and loss of the original cultures.

On the basis of these criteria it was presumed that the fish had Columnaris disease and all the trout yearlings were given a single copper sulphate solution dip (1:2000 for 45-75 seconds). The brown trout yearlings were given a 10 day feeding treatment of oxytetracycline (dose 50 mg per kg of body weight per day) and the rainbow trout had sulphamerazine mixed into the food (dose 220 mg per kg of body weight per day for three days, then 145 mg per kg of body weight for the next seven days).

The two year old and three year old fish did not require any treatment.

Within three weeks the infection was completely controlled with an estimated loss of 8% brown trout yearlings and 1-2% rainbow trout yearlings.

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FISH STUDIES

THE EFFECTS OF JUVENILE BEHAVIOUR ON THE DYNAMICS OF LAKE EUCUMBENE TROUT POPULATIONS

(abstract only)

by

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Population estimates indicate that Lake Eucumbene is now carrying twice as many brown trout as rainbow trout, and a major cause of this imbalance is differential mortality in the first year of life of the fish.

The great majority of spawning streams is shared by both species. The territorial behaviour exhibited by the earlier hatching brown trout fingerlings was shown to result in displacement of rainbow trout juveniles from the natal streams; brown trout juveniles are significantly larger than their rainbow counterparts during this period of maximum interspecific competition. Fingerlings were shown to incur higher mortality under lentic than under lotic conditions and the early entry of rainbow trout juveniles into the lake results in heavier mortality compared with brown trout juveniles, the majority of which remain in the natal creeks for a year.

The increased interspecific competition is due to the heavy spawning pressure placed on available streams by the large lentic trout population which became established after creation of the impoundment.

It is interesting to note that immediately prior to the formation of Lake Eucumbene in 1957, the resident trout population of the Eucumbene River (the major spawning stream) comprised 88% rainbow trout and 12% brown trout whereas in 1971 the composition was 3% rainbow trout and 97% brown trout.

THE INFLUENCE OF RIVER LEVEL ON SPAWNING OF THE MURRAY COD (*MACCULLOCHELLA MACQUARIENSIS*)

(Recorded here by title only)

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REPRODUCTION IN SOME TROPICAL AUSTRALIAN FRESHWATER FISHES

(Recorded here by title only)

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FRESHWATER FISHES OF THE NORTHERN TERRITORY

(Recorded here by title only)

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ADAPTATIONS OF A CENTRAL AUSTRALIAN GOBIID FISH[+]
(abstract only)

by

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[+]A Summary of material to be presented in a series of forthcoming papers (Glover, unpublished data).

The desert goby *Chlamydogobius eremius* (Zietz) is endemic to the Lake Eyre drainage system where it appears to be adapted specifically to living in the interesting environment formed by the streams of artesian springs and bores whose saline waters are known to be subject to fluctuations in composition, concentration and temperature and to periods of considerable drying up. The species possesses a series of interesting adaptive features, which enable it to establish itself and survive in a tenuous, fluctuating and unstable environment.

PHYSIOLOGY

The ability of this small fish to inhabit widely varying and fluctuating environments may be explained, in part, by its extreme physiological lability as evident by the wide range of physical environmental conditions under which it is known to occur and in which it can survive under experimental conditions.

Thus, in the field the fish has been taken in waters whose salinities range from 200 to 12,500 p.p.m. and in the laboratory it has tolerated periods of up to 27 days direct immersion in distilled water and periods in excess of 60 days in seawater exceeding 37,000 p.p.m. It is clear that larger permanent aquatic habitats (artesian and meteoric) as well as the small ephemeral ones are subject, at least temporarily, to fluctuations of salinity due to climatic factors. Within individual artesian waters substantial salinity gradients and differences in ionic content have been found along stream paths. In addition the composition of outflowing artesian water may and does change significantly over a varying period of time, sometimes quite dramatically. Salinity in fact is probably in a constant state of flux. The fish is therefore subject to, and clearly successfully adapted to, wide fluctuations in water salinity and composition in time and space, even in apparently permanent bodies of water.

Regarding temperature, the fish has been taken at one locality in water of 9°C in winter months and in water of 40°C in summer months. The fish has been found to take advantage of refuges provided by thermal gradients, particularly in summer months, when sections of some water masses heat to lethal levels. Water temperature transects made between open artesian streams and vegetated shallows have demonstrated the existence of pronounced lateral thermal gradients in which temperature drops with increasing distance into vegetation stands where fish tend to congregate when open water temperatures approach lethal levels. Vertical thermal gradients have also been detected, between the surface of artesian stream beds and immediately beneath, in the fine silt of which sections of the beds are characteristically formed. Sub-bed temperatures are invariably less (by as much as 10°C) than bottom water temperature, at least down to depths of 10 cm, especially in summer months. Fish have been found buried in relatively cool bed silt when water temperature immediately above has been close to their maximum tolerance level. However, laboratory observations suggest that the fish do not actively burrow into silt but tend rather to settle passively into bed surfaces.

The species acclimates to seasonal changes in water temperature and to simultaneous thermal gradients existing in streams. In the laboratory it acclimates rapidly to large and sudden increases in water temperature – within 10 hours to rises of 10 – 15°C. Since the artesian habitat of the fish characteristically exhibits substantial lateral thermal gradients over relatively short distances (e.g. 21°C over 60 cm), as well as pronounced vertical gradients, it is obviously to the species' advantage to tolerate and readily acclimate to sudden changes in temperature.

There appear to be no significant thermal or ionic differences between inhabited and non-inhabited water masses within the Lake Eyre drainage system to account for the occurrence or absence of the species.

Regarding dissolved oxygen levels, data are limited. Nevertheless fish have been observed in waters with levels as low as 0.8 p.p.m. and commonly at concentrations of 2–4 p.p.m. Under conditions of presumed low dissolved oxygen tension the fish has been observed to lie in shallows almost completely out of water apparently surviving by aerial respiration. Fish maintained in tanks during warm weather and exposed to direct sunlight have been observed to lie for prolonged periods with the forepart of the body out of water resting on the surfaces of emerging clumps of vegetation and silt. Active opercular movements suggested that aerial respiration was being performed in response to depleted oxygen levels.

DISPERSAL MECHANISMS

The marked morphological uniformity between populations throughout the known range of the species suggests that recent dispersion has occurred and/or that there is sufficient gene flow between populations to ensure that minimal morphological divergence has occurred. In view of the successful establishment of populations in various pools and streams associated with artesian bores since European settlement it is apparent than an efficient dispersal mechanism is operating and that so long as it continues to operate the species will probably retain its morphological uniformity and consequently its monotypic status. Furthermore this may explain the retention of relative non-specialization by the fish (within the context of the Lake Eyre basin habitats) and consequently its wide spectrum of environmental tolerance.

The occurrence of small isolated adult populations in the ephemeral bodies of water following seasonal rains suggests that transport by floodwaters is probably an important, possibly the only, means by which dispersion of stock is effected from centres of permanent population. The extensive and substantial floodings and the extensive areas of low topographic relief characteristic of the central Australian region are certainly conducive of efficient and frequent dispersion of aquatic fauna by floodwaters.

The fact that all recorded occurrences, both permanent and transitory, are restricted to within the Lake Eyre drainage system supports the postulate that dispersion is probably effected exclusively by flood-waters and therefore can only occur within the drainage area. The apparent general absence of the species from all but a few of the many bore sites east of Lake Eyre and from some bores and springs in close proximity to large permanent populations, all of which are visited frequently by various water birds, and its apparent absence outside the Lake Eyre drainage basin, tends to negate the proposition that dispersion is achieved, even in part, by some form of aerial transport e.g. transportation of juveniles or adults via thermals (willy-willies) or of fertilized eggs via attachment to the feet of water-wading birds. There are probably other factors, especially of an aquatic

chemico-physical nature, that prevent the establishment of breeding populations of *C. eremius* in artesian waters outside the central Australian region but these are probably secondary to the limitations imposed on distribution by the mode of dispersion.

In the light of these observations it appears that there is no obvious factor (apart from possibly topographical barriers restricting the flow of floodwaters) to restrict the occurrence of *C. eremius* to any of the scattered waters of the Lake Eyre system and that there are probably frequent movements between populations. The occurrence of only one species over such a wide area is therefore understandable.

COMPETITION AND PREDATION

The other fish most typically found in association with *C. eremius* or inhabiting similar waters in the Central Australian region are the equally small craterocephalids which do not appear to pose a significant predatory threat. The primary predatory forces seem likely to be aquatic birds and dytiscid beetles, but in the case of birds this influence appears to be reduced to a minimum as a result of the fish's strong negative phototactism and consequent preference to inhabit vegetation cover rather than open water. In addition the fish's demonstrated ability to change colour rapidly to conform cryptically with its background undoubtedly enhances its ability to avoid the attention of potential predators.

FEEDING

With respect to diet, the fish is omnivorous and utilizes as food that flora and fauna which is present in virtually every artesian waterway in the Lake Eyre basin, principally filamentous algae *(Spirogyra)* and a range of small animals (insects, ostracods, copepods). Thus there is no apparent specialization in diet or feeding habit to restrict the fish's occurrence within the inhabited range. Because olfactory/gustatory senses have been shown to be the primary means by which the species locates food the fish is readily able to feed at night as well as in the day, as in fact it does, thus reducing its dependence on daylight feeding and possibly its chances of exposing itself unnecessarily to predators. This ability to locate food by means of non-visual senses is also a suitable adaptation to a fish which spends a considerable amount of time under the cover of vegetation, in particular the filamentous algae which constitutes part of and harbours some of the animal components of its diet.

SUMMARY

C. eremius inhabits an environment in which salinity, ionic composition, temperature and possibly dissolved oxygen may grade and fluctuate rapidly over a wide range. It is clear that the fish is well adapted, both physiologically and behaviourily, to acclimate to or survive, at least temporarily, such stresses. Furthermore it appears to utilize frequent and extensive floodwaters as a means of ensuring regular dispersion, thereby maximising its ability to maintain or establish populations as others are threatened. The species is adaptive and opportunistic, features which have ensured its survival in waters subject to extreme change and which have probably ensured its integrity as a single species.

COMPARISON OF STRAINS OF *SALMO GAIRDNERI* RICHARDSON FROM NEW SOUTH WALES, VICTORIA AND WESTERN AUSTRALIA

by

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INTRODUCTION

The hatchery brood stock of rainbow trout which is maintained at the Pemberton Hatchery, Western Australia, occasionally suffers severe reduction in numbers during the hot dry summers in years when the water supply fails (e.g., in 1961 and 1970). Since re-establishment of the stock from survivors takes two years (female rainbow trout at this latitude – 34°27'S – mature in the second year of life, and ripen in early winter), the heavy mortality in 1970 necessitated importation of eyed ova from New South Wales and Victoria to fulfil annual stocking commitments in the local fishery later that year.

The presence of young fish from three different climatic regions of Australia at Pemberton provided an opportunity to investigate whether there was any real biological advantage in maintaining a local brood stock as opposed to annual importation of ova. The summer climate of inland waters here is the most important component of environment limiting the distribution and abundance of trout (Morrissy, 1972). The advantage in maintaining a strain of local fish (the first introduction of this species to Western Australia occurred in the mid 1930's) for stocking purposes, rather than using the imported progeny of parent fish living in more temperate parts of Australia has been thought, *a priori,* to be their superior ability to survive the summer in the south-west of Western Australia. This opinion was supported by the fact that after "eyed" ova had been exported from Western Australia to the Tasmanian Inland Fisheries Commission in 1969, the Commissioner of that body advised . . . "the Commission noted that the Western Australian strain of rainbow trout stood up to the stress of higher temperature at the Salmon Ponds better than did the Tasmanian strain of rainbow trout" (Lynch, pers. comm.).

MATERIALS AND METHODS

The New South Wales fish were obtained from wild parents trapped in tributaries of the Eucumbene River and the ova were held at the Jindabyne Hatchery until "eyed". The Victorian fish were progeny of the domesticated brood stock maintained at the Snobs Creek Hatchery, Alexandra.

The Western Australian rainbow trout are the direct descendents of fish imported from Ballarat, Victoria in 1936, and to a lesser extent in 1937, which were released in streams near Pemberton (Pemberton-Warren Trout Acclimatization Society, 13th Annual Report, 1943). By 1946 a brood stock of descendents of these wild fish was established in a hatchery at Pemberton (Pemberton-Warren Trout Acclimatization Society, 16th Annual Report, 1946).

As an indication of the ability of the fish to survive summer conditions the resistance time (geometric mean of individual times of survival) was estimated for different constant lethal temperatures (see Fry, 1956). Test temperatures were maintained at the selected value with ± 0.1°C variability using a Techne Tempunit in an insulated waterbath. Samples of fish were tested in a stainless steel container, suspended in the bath, containing water from the nearby Lefroy Brook (pH = 7.0, total dissolved solids 160 p.p.m., NaCl = 110 p.p.m. and Ca^{++} = 5 p.p.m.). The test water was continually aerated with medical oxygen. The fish were not acclimated to a constant favourable water temperature prior to the tests because of lack of suitable facilities, but each strain was subject to the same conditions and the water temperatures for the week prior to the tests were taken as a measure of the level of acclimatization. The test fish were fed four times daily with liver as fry, twice daily as fingerlings, and only once daily during the period of testing.

Victorian and Pemberton fish age 0+ were tested in November 1970 at 29°C, 28°C and 27°C following acclimatization to temperatures around 14°C. New South Wales, Victorian and Western Australian fish were tested in late February-early March 1971 at 30°C, and 29°C and 28°C with prior acclimatization to temperatures around 21°C. Tests involving resistance times in excess of 4 hours (i.e., at still lower lethal high temperatures) could not be attempted because of difficulty in maintaining test temperatures with sufficient accuracy, without continual checking, under conditions of widely fluctuating air temperatures.

Fork lengths (L) of the test fish were recorded, and in the second series of tests weighing (W) also allowed calculation of condition factors ($K = 100\,W/L^3$) maintained by the fish through the summer. Some meristic characteristics were also recorded from small samples of the test fish, viz. number of vertebrae, number of gill rakers on the right anterior arch, number of anal fin rays, and number of parr marks.

RESULTS

The responses of the fish in each test batch are normalized by taking logarithms of time to death and summarized by the geometric mean resistance time and asymmetric 95% confidence limits (Table I). The summarized data are customarily graphed by plotting resistance time on the abscissa and the corresponding lethal temperature on the ordinate (Fig. 1). However, to test whether significant differences occur between the strains of trout it is more realistic to consider temperature as the determining variable and to compare the constants, slopes and intercepts, of the respective linear regression equations.

The regression equation $\log_{10}$ TIME (time to death of individual fish in minutes) on TEMPERATURE (the constant lethal temperature in °C) was computed for each batch of fish at the two acclimatization levels. For acclimatization to approximately 14°C:–

Victorian fish $\log_{10}$ TIME = 11.2062 – 0.3354 TEMPERATURE
variance (S^2) = 0.004438

Western Australian fish

$\log_{10}$ TIME = 11.43610 – 0.3417 TEMPERATURE
(S^2) = 0.002744

TABLE 1

RESISTANCE TIMES (GEOMETRIC MEAN TIME TO DEATH = L.D. 50) OF *S. gairdneri* STRAINS AT CONSTANT LETHAL WATER TEMPERATURES FISH AGE 0+, (n = sample number of test fish; parentheses enclose the geometric 95% confidence limits of the sample)

	New South Wales	Victoria	Western Australia
A. Experiments, 9-11 November 1970. Acclimatization water temperatures during preceding week:– Mean, 14.2°C Range, 13.3°C – 15.0°C			
At 29°C	–	30.78 mins. (26.2 – 36.1) n = 10	34.99 mins. (31.7 – 38.6) n = 10
28°C	–	62.59 (58.4 – 67.1) n = 11	68.47 (64.2 – 73.0) n = 11
27°C	–	154.7 (142.7 – 167.7) n = 14	168.8 (158.9 – 179.3) n = 10
B. Experiments, 28 February – 5 March 1971. Acclimatization temperatures:– Mean, 21.0°C Range, 19.4°C – 22.5°C			
At 30°C	34.11 mins. (31.1 – 37.4) n = 11	35.85 mins. (32.8 – 39.2) n = 12 *32.43 mins. (28.3 – 37.1) n = 14	32.03 mins. (27.3 – 37.6) n = 10 *33.60 mins. (31.3 – 36.0) n = 10
29°C	75.25 (69.1 – 81.9) n = 10	81.45 (72.7 – 91.2) n = 10	84.80 (79.7 – 90.2) n = 10
28°C	174.3 (168.1 – 184.1) n = 15	169.4 (159.3 – 180.1) n = 12	195.8 (181.1 – 211.7) n = 11

* Result of separate test on a second batch of fish

For acclimatization to approximately 21^0C:–

New South Wales fish

$$\log_{10} \text{TIME} = 12.1817 - 0.3551 \text{ TEMPERATURE}$$
$$(S^2) = 0.002602$$

Victorian fish

$$\log_{10} \text{TIME} = 12.0173 - 0.3493 \text{ TEMPERATURE}$$
$$(S^2) = 0.004618$$

Western Australian fish

$$\log_{10} \text{TIME} = 13.2140 - 0.3898 \text{ TEMPERATURE}$$
$$(S^2) = 0.003868$$

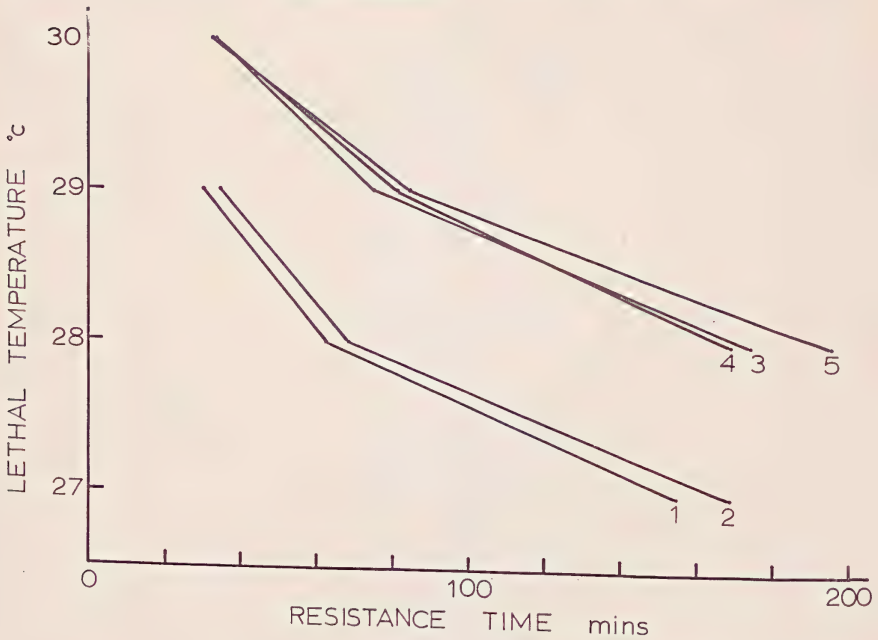

Fig.1. Resistance times of *Salmo gairdneri* at constant lethal high temperatures.

1. Rainbow trout, age 0+, from Snob's Creek Victoria:
   tested at Pemberton in Nov. 1971;
   acclimatization water temperatures 13.3–15.00^{0}C
2. Rainbow trout, age 0+, from Pemberton W.A.
   tested at Pemberton in Nov. 1971;
   acclimatization water temperatures as above
3. Rainbow trout, age 0+ from Jindabyne-Eucumbene,N.S.W.
   tested at Pemberton in Feb – March 1971;
   acclimatization water temperatures 19.4–22.5^{0}C
4. Rainbow trout, age 0+, from Snob's Creek Victoria;
   tested at Pemberton in Feb – March 1971;
   acclimatization water temperatures as above
5. Rainbow trout, age 0+, from Pemberton, W.A.;
   tested at Pemberton in Feb – March 1971;
   acclimatization water temperatures as above

The intercepts of the regression equations for Victorian and Western Australian fish at the lower acclimatization level were significantly different (at the 0.1% level). The Western Australian fish were consistently more resistant over the range 29° to 27°C. At the higher acclimatization level the slope of the regression equation for the Western Australian fish differed significantly (at the 5% level) from the pooled slopes of the equations for the imported fish. The three strains of trout did not differ at 30°C but the Western Australian fish were increasingly more resistant towards lower lethal temperatures than the other strains.

Table 2 shows the resistance times of the trout, calculated from the regression equations. The incipient lethal temperature is assumed to lie in the region of 25-26°C. Extrapolation is permissable since the log TIME-TEMPERATURE relationship has been found to be linear down to the incipient value for many species of fish (Fry, 1956).

TABLE 2

MEAN RESISTANCE TIMES (MINS) OF RAINBOW TROUT CALCULATED FROM REGRESSION EQUATIONS (See text)

Acclimatization Temperature	Source of Trout	30°C	29°C	28°C	27°C	26°C	25°C
14°C	VICTORIA	13.9	30.1	65.2	141.1	305.4	661.1
	WESTERN AUSTRALIA	15.3	33.6	73.8	162.0	355.9	781.6
21°C	NEW SOUTH WALES	34.3	76.6	173.5	393.1	890.4	2017
	VICTORIA	34.6	77.4	173.0	386.6	864.0	1931
	WESTERN AUSTRALIA	33.2	81.3	199.5	489.5	1201	2947

There was no significant difference between the mean lengths of Victorian and Western Australian fish tested in November 1970, and in the late summer of 1971 (Table 3). However, at the latter time the New South Wales fish were significantly smaller in size than the other two stocks. Western Australian fish at that time also had a significantly higher condition factor (K) than Victorian fish, while New South Wales fish were significantly poorer in condition than either Victorian or Western Australian fish. The hatchery assistant in charge of the stocks noticed that the New South Wales fish were not feeding well during the summer and gave some of these fish more living space but without a favourable response. A higher percentage of progeny of the wild N.S.W. fish had orange coloured paired fins compared with those of the hatchery brood fish which are fed carotenoid deficient diets (particularly in the case of Western Australian fish).

Early development of the strains of fish occurred under very different temperature conditions (Table 4) with New South Wales embryos exposed to the lowest temperatures and Western Australian embryos to the highest values. There did not appear to be any linear correlation between these conditions and values of meristic characteristics. Vertebrae number has been experimentally established by Garside (1966) as negatively correlated with water temperature, i.e. positively development time. Gill raker, anal fin ray, and parr mark counts were slightly higher for Victorian fish in

TABLE 3

CHARACTERISTICS OF *S. gairdneri* STRAINS, FROM THREE LOCALITIES IN AUSTRALIA, WHICH WERE HELD AT PEMBERTON, WESTERN AUSTRALIA, DURING 1970-1971.

	New South Wales	Victoria	Western Australia
Sources of ova	Wild fish captured in tributaries of Eucumbene R.	Snob's Creek Hatchery Brood Stock domesticated since 1955	Pemberton Hatchery Brood Stock domesticated since 1946
Dates of Stripping of Ova	Probably the last week in August 1970. Embryos held at Jindabyne Hatchery.	Not Known	18.iv.1970
Dates of hatching at Pemberton	23-29.x.1970 (received 22.x.1970)	6-13.viii.1970 (received 4.viii.1970)	12-22.vii.1970
Nov. 1970 Mean Fork Length cm (S.D.)	–	4.95 (0.528)	4.66 (0.469)
		No sig. difference between means at the 5% level of probability	
Feb. March 1971 Mean Fork Length cm (S.D.)	4.20 (0.598)	7.11 (0.932)	6.82 (1.062)
		N.S.W. versus Vic. + W.A. sig. ***	
Mean K (S.D.)	1.06 (0.099)	1.16 (0.078)	1.20 (0.112)
	N.S.W. versus Vic. or W.A. sig. ***	Vic. v W.A. sig.*	
Mean count of parr marks (S.D.)	9.2 (1.76)	10.6 (0.98)	9.5 (1.22)
% with orange paired fins	88.9	58.3	46.3
No. of vertebrae (5 fish)	mean 62.6 (61-64)	mean 61.6 (60-63)	mean 62.8 (61-64)
No. of anal fin rays	mean 14.6 (14-15)	mean 15.2 (15-16)	mean 15.0 (14-16)
No. of gill rakers	mean 17.8 (15-19)	mean 18.4 (17-20)	mean 17.6 (15-19)
Av. Monthly Water Temp. (°C) at Source Hatchery 1970	Max Min Av.	Max Min Av	Max Min Av.
June	6.6° 3.3° 5.0°	9.4° 4.4° 7.2°	11.4° 10.6° 11.0°
July	3.4° 2.0° 2.7°	8.9° 5.0° 7.0°	11.7° 10.1° 10.9°
August	4.1° 2.8° 3.5°	8.9° 3.3° 6.1°	11.3° 9.7° 10.5°
Sept.	5.0° 3.3° 4.2°	9.4° 5.0° 7.0°	12.8° 11.0° 11.4°
Oct.	8.0° 5.6° 6.7°	12.2° 5.6° 8.9°	14.5° 13.1° 13.8°

("t" values for difference between means
* Significant at 5% level of probability
*** Significant at 0.1% level of probability)

the mid-range of incubation temperatures and vertebrae counts were slightly lower for these fish compared with New South Wales and Western Australian fish. Seymour (1959) found less vertebrae in young *Oncorhynchus* that had developed in the middle of the favourable temperature range of 3.9°C – 16.7°C and more at the extremes; the reverse was found for fin rays. Mottley (1937) found that there was wide variability in the vertebrae number of trout due to a variety of factors.

The ranges of values of meristic characters for the Australian fish reported here are small compared with the ranges reported in the literature:– parr marks, 8-15 (Australian fish are in the lower range); vertebrae, 57-66 (Australian fish are in the mid range); gill rakers, 16-21 (low); and anal fin rays, 9-16 (high).

Overseas values for *S. gairdneri* are from Jordon and Evermann (1896), Needham and Gard (1959), Bidgood and Berst (1967), and Northcote *et al* (1970).

TABLE 4

MAXIMUM WATER TEMPERATURE (°C) AT THE SOURCE HATCHERIES DURING "SUMMER", 1970

(1) Average water temperature for month. (2) Average maximum water temperature for month. (3) Highest daily maximum water temperature for month.

	New South Wales			Victoria			Western Australia		
	(1)	(2)	(3)	(1)	(2)	(3)*	(1)	(2)	(3)*
November	10.9	13.9		10.6		15.6	15.6	16.3	18.3
December	15.7	18.0		13.3		17.8	18.9	19.6	21.7
January	13.3	14.4		13.3		18.3	22.4	23.0	24.4
February	17.0	19.0		14.2		20.0	21.7	22.0	23.3
March	14.0	16.4		12.5		20.0	20.1	20.4	22.0

* Measured at Hatchery inlet of water supply pipeline

** Measured at Hatchery inlet of water supply pipeline. Pond temperatures exceeded these values by 1 2/3°C on the average.

DISCUSSION

The lethal temperature data fall into two distinctive groups illustrating the marked influence of seasonal temperature acclimatization (Fig. 1). The data of Bidgood and Berst (1969) for Great Lakes rainbow trout (acclimated to 15°C) and Morrissy (1967, 1971) for Snobs Creek rainbow trout (17.8-21.8°C) are similar to the results for the lower acclimatization temperatures.

At the higher acclimatization temperatures (>20°C) the coincidence of the resistance times of the New South Wales, Victorian and Western Australian strains of trout at 30°C indicates, in contrast to the divergent results at lower lethal temperatures, that a ceiling has been attained in the ability of the fish to further increase resistance times at lethal high temperatures. It is interesting to note that the heat capacity of pure water exhibits a minimum at 30°C (Hutchinson, 1957) and to speculate whether *Salmo* (and perhaps other "cold-water" fish) are unable, genetically, to overcome this temperature barrier in an evolutionary sense because of this property of water.

The superior acclimatization by Western Australian fish to high summer water temperatures appears to result in higher resistance times at lower lethal high temperatures only. Of course the latter are more realistic ecologically, i.e. likely to occur more frequently in nature, and the hatchery brood stock in Western Australia has been exposed to severe natural selection in this regard for many years (see comparative summer temperatures, Table 4). This brood stock has also been consistently exposed to natural selection by low oxygen levels, excessive accumulation of metabolic and waste products, poor food supply, and crowding. In addition the largest individuals of a year class are artificially selected for future breeding (Pemberton-Warren Trout Acclimatization Society, 17th Annual Report, 1947).

Donaldson and Olson (1956) consider rainbow trout to be particularly suitable for selective breeding in hatcheries for earlier maturity, more rapid growth, higher fecundity, resistance to disease, changed season of spawning, and increased tolerance to warm water, having established marked changes in a line of fish selected over a period of 23 years. However, McCauley (1958) was unable to establish a significant difference between the lethal temperature responses of two hatchery brood stocks of *Salvelinus fontinalis* (Salmonidae) where one strain "was maintained and thrived in water of a temperature somewhat higher than the range typical . . . of this species." A difference was found only between two morphologically recognised wild subspecies of *Salvelinus.*

The question that must therefore be asked is whether the differences reported here, for geographic strains of rainbow trout, are the outcome of genetical adaptation to different climates or merely phenotypical variations due to their dissimilar early embryonic conditions.

Meristic counts on the three strains of Australian trout indicated that embryonic conditions had resulted in differences which were not, however, marked compared with the reported wide variability in this trout species. A crucial indicator of the unimportance of embryonic temperature conditions in influencing the physiological response to lethal temperature later in life is the lack of a significant difference between the response of New South Wales and Victorian fish in this regard (although the latter domesticated fish performed better under routine rearing conditions). This result would seem to indicate that the W.A. fish differed genetically from the other two strains, and, in addition, Craigie (1963) reported that the thermal resistance of two groups of *S. gairdneri* experimentally hatched and reared at 7.5°C and 15°C, respectively, did not differ.

In view of Donaldson and Olson's (1956) report it is not improbable that a genetical difference could have been established at the Pemberton Hatchery over 25 years in a continuing line of brood stock. However, F.E.J. Fry (pers. comm. 1971) questions which genetic factor might have been so rapidly selected. Of especial interest is the very low ionic concentration of the Pemberton Hatchery water, e.g. (Ca^{++}) = 5 p.p.m., because McMartin, working in a New England state, reported that rainbow trout did not reproduce at about 3 p.p.m. of Ca (Fry, pers. comm.). Halsband (1953) found that addition of Ca and Mg to test water increased lethal temperature resistance of rainbow trout. Craigie (1963) found that rainbow trout reared in soft water performed better than ones reared in hard water while resistance to lethal temperatures was higher in a saline test medium than in a soft one for both groups. However, the hardness of the environmental water during the embryonic development and alevin growth of his rainbow trout did not have any significant influence upon their thermal resistance

later. Thus the imported New South Wales and Victorian fish in the experiment reported here would have been acclimated to the soft Pemberton test water. Fry (pers. comm.) suggests that any difference between the results of this experiment and one made in the future using ova from elsewhere but fertilized here would be the result of selective survival (of incubating embryos) rather than greater acclimation. It is interesting to note that the water supply to the Snobs Creek Hatchery in Victoria is also very "soft" (pH 6.8, total dissolved solids = 36 p.p.m., Ca (as $Ca\ Co_3$) = 1.7 p.p.m., Mg = 1.0 p.p.m. and C1 = 3.2 p.p.m.[Affleck, 1952]) so that low ionic concentration *per se* acting on the embryos cannot be the supposed selective factor. Since the early half of ovarian development of the trout proceeds through the "summer" months it is reasonable to propose, from all the above considerations, that a selective interaction of low ionic concentrations and high water temperatures acts on the adult fish, particularly with regards to ovarian development.

With this hypothesis in view a nucleus of the Victorian strain has been retained at Pemberton for future testing of their progeny.

CONCLUSIONS

Progeny of the Western Australian hatchery brood stock were shown to be superior, phenotypically, in responses at high water temperatures, to the imported progeny of parent fish living in cooler parts of Australia. This knowledge is of practical value for maintaining the Western Australian trout fishery which requires annual liberations of hatchery fish.

The Western Australian *Salmo gairdneri* may differ genetically from other rainbow trout in Australia in responses at high water temperatures. If further work confirms this suggestion, maintenance of such a hatchery strain would also be valuable elsewhere in Australia for stocking marginal trout waters where selection of fish by summer heat stress cannot be perpetuated by natural reproduction.

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A NOTE ON THE TOXICITY OF "PEST STRIPS" CONTAINING THE ORGANOPHOSPHORUS INSECTICIDE DICHLORVOS (DDVP) TO THE MOSQUITO FISH, *GAMBUSIA AFFINIS* (CYPRINODONTIFORMES: POECILIIDAE)

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INTRODUCTION

Dichlorvos, or DDVP, the active insecticidal ingredient of *Shelltox* "Pest Strips", has previously been shown to be toxic to fish when dissolved directly at relatively high concentrations in the water containing them (Sreenivasan and Swaminathan 1967; Konar 1969; Eisler 1970).

The simple experiment described below was carried out in response to an enquiry from an aquarist concerning the possible toxic effects on fish from the use of such "pest strips" for insect control in an aquarium room. Its main aims were to accelerate solution of the dichlorvos artifically from the air around the pest strip to the aquarium water, to determine if this compound reached dissolved concentrations that were lethal to fish, and if so to note its effects on their behaviour prior to death.

MATERIALS AND METHODS

The *Shelltox* "Pest Strip" (Shell Chemical Co.) is composed of a plastic-resin strip impregnated with 18.6% of the organophosphorus insecticide dichlorvos, or DDVP (*0,0*-dimethyl *0*-2, 2-dichlorvinyl phosphate), together with 1.4% of related compounds. Controlled vaporisation of the insecticide (trade name *Vapona*) from the resin occurs to the surrounding air and is claimed by the manufacturer to be effectively insecticidal, "for up to 3 months . . . in an average size room – about 1,000 cubic feet". Release of dichlorvos vapour from a freshly opened pest strip occurs at a rate of about 10 mg/1 for the first week and averages 8 mg/1 for the first 30 days. During the second and third 30 day periods the release rate drops to 5 and 3 mg/1, respectively (Tinker 1972).

The fish species chosen for this preliminary toxicity experiment was the mosquito fish, *Gambusia affinis,* a small, introduced, freshwater, live-bearing poeciliid. This exotic species, which is now well established throughout many areas in Australia, has a reputation for "hardiness" under a variety of different physical and chemical environmental conditions, and was recommended as a test animal for comparative toxicological studies on the effects of insecticides on fishes by Ali and Giurgis (1967). The fish used in the present study were captured in Deep Creek, which enters Narrabeen Lakes, about 20km north of Sydney.

For the experiment two small perspex aquaria, each containing approximately 8 l of tap-water, were left to stand for 2 days, after which time 12 fish between 2.5 and 3.0 cm long were added to each tank (on 7-v-1971). Aeration was delivered to the aquaria through air stones from a central supply, and on the fourth day (11-v-71) after addition of the fish (day 1 of the experiment) the aeration to one of the acquaria was diverted through a sealed 5 l polythene jar containing a freshly opened *Shelltox* "Pest Strip"; the other was retained as a control. The fish were fed daily with small amounts of a dried trout-food preparation (Ewos), and the aquaria examined twice (at approximately 9.00 am and 5.00 pm) each weekday for 60 days. The appearance and behaviour of the control and experimental fish were compared, and the numbers of dead and visibly "sick" fish noted on each occasion. Although water temperature was not monitored regularly during this experiment, a number of 9.00 am temperatures were taken during the study period, and these ranged from about 13 to 16°C.

RESULTS

The results of the experiment are graphed in Fig.1, in which the numbers of surviving fish in each of the control and experimental groups at 9.00 am each weekday after commencement of the experimental treatment are shown.

It can be seen from this figure that whereas only 3 of the 12 control fish had died by the end of the 60 day period, all 12 in the experimental group had died by the 37th day. The nine remaining control fish were still alive by the 80th day.

In the case of each of the 3 control fish which died, no visible symptoms were noted on the days prior to death. In the case of the experimental group, however, one fish was observed to show intermittent symptoms of muscular paralysis in the caudal (but not the pectoral) region from the first day of the experiment; this was almost certainly the fish found dead on day 15.

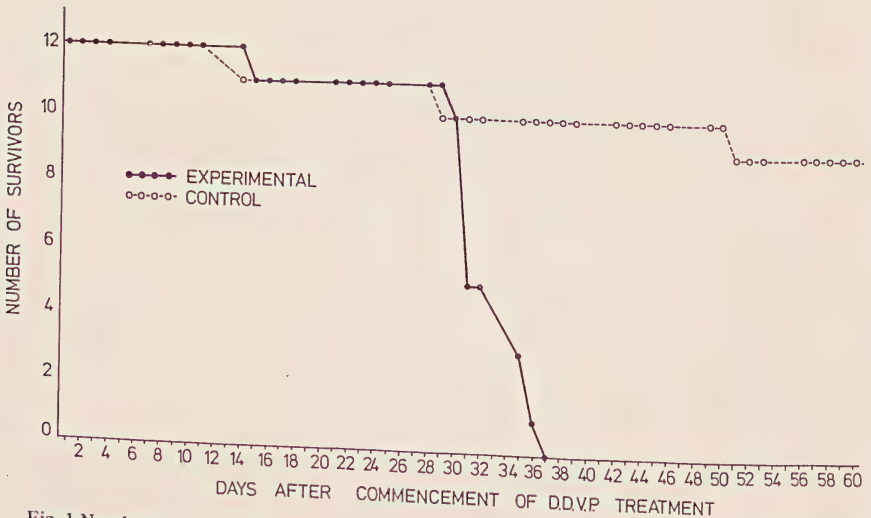
NUMBER OF SURVIVORS
12
10
8
6
4
2
0
EXPERIMENTAL
CONTROL
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50 52 54 56 58 60
DAYS AFTER COMMENCEMENT OF D.D.V.P. TREATMENT

Fig. 1 Numbers of surviving fish in control and experimental (dichlorvos treated) groups at 9.00 a.m. each weekday after commencement of experimental treatment.

On the 21st day of the experiment the reactions of the remaining fish in the experimental group were first noticed to be less intense than those of the controls on approach of the aquaria by an observer. This observation was confirmed by comparing the fall off in the "darting" response of the fish when the sides of the control and experimental aquaria were tapped sharply a number of times in succession. This "reaction intensity" became noticeably impaired (although no attempt was made to quantify it) in the experimental compared with the control group during the following week, until a noticeable stiffness of the pectoral fins, accompanied by a slight darkening of pigmentation, apparent symptoms of malnutrition, and a tendency to swim slowly at the water surface were observed in a number of the experimental fish on day 29. The numbers of experimental fish showing increasing degrees of these symptoms (Plate 1), especially an apparent paralysis of the pectoral fins in a position perpendicular to the body axis and of the anterior (but not the caudal) body musculature, and then a tendency to lie at the surface on one side, increased rapidly over the following week, during which period the remaining 11 experimental fish died in rapid succession (see Fig. 1).

Thus, excluding the first experimental fish which had died by day 15 and showed different symptoms prior to death, the remaining fish in the experimental group all displayed similar symptoms and died within the 8 days between the 29th and 37th days after commencement of the experimental treatment, presumably due to the chronic effects of dichlorvos toxicity.

Plate 1. Live experimental fish (left) showing apparent paralysis of pectoral fins, darkening of pigmentation, and symptoms of malnutrition after 30 days of dichlorvos treatment, compared with normal, live, untreated control fish (right).

DISCUSSION

That the organophosphorus insecticides are generally less toxic to fishes than the organochlorine group (Henderson and Pickering 1958), has recently been confirmed by Eisler (1970), who found that of 12 insecticides tested, endrin and DDT (organochlorine) were the most toxic and methyl parathion and DDVP (organophosphorus) the least toxic to the 7 species of fishes treated. The concentrations of DDVP fatal to 50 percent of each of the 7 species in 96 hours (96 h. LC_{50}) ranged from 225 to 2,680 micrograms per litre (ppb), compared with 0.05 to 3.1 ppb for endrin. The 96 h. LC_{50}'s for two species related to *G. affinis (Fundulus majalis* and *F. heteroclitus*; Cyprinodontiformes: Cyprinodontidae) were 2,300 and 2,600 ppb, respectively.

Although the primary action of organochlorine insecticides is thought to be directly upon the nervous system (in both vertebrates and invertebrates), the effects of the organophosphorus group is explained on the basis of inhibition of cholinesterase activity (O'Brien 1967). The observed effects of tetany of the muscles controlling the pectoral fins in the experimental fish in the present experiment may thus be due to the anti-cholinesterase activity of the concentration of DDVP absorbed by the fish from the water after 29 days. However, due to the lack of suitable laboratory facilities, and the fact that organophosphorus insecticides are rapidly degraded in water, the concentrations of DDVP in the experimental system could not be determined.

From the results of the above experiment, even although the treatment was rather extreme, the use of *Shelltox* "Pest Strips", at least for extended periods, for the control of insects in rooms containing fishes cannot be recommended as safe.

Because of the differential toxicity of DDVP and other organophosphorus insecticides to different species of fishes, and because of their rapid decomposition in water, the possibilities for their use in aquaculture as selective fish toxicants and for the eradication of undesirable fish from ponds and lakes have been stressed by Sreenivasan and Swaminathan (1967) and Konar (1969).

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PRELIMINARY NOTES ON AUSTRALIAN BLEPHARICERIDAE (DIPT.)

(Recorded here by title only)

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REPRODUCTION IN THE FRESHWATER SHRIMP, *MACROBRACHIUM*

(Recorded here by title only)

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A RECENT STUDY OF SOME FOSSIL FRESHWATER BIVALVES OF LIMNOLOGICAL INTEREST

(Recorded here by title only)

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PHYSIOGRAPHY OF NINE SELECTED VICTORIAN LAKES

(abstract only)

by

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Seven of the lakes lie on the volcanic plains of the Western District. Five are maars and each has steeply shelving littorals and a flattish floor. Purrumbete is 45m deep, Bullenmerri 66m, Gnotuk 18.5m, East Basin 12m and West Basin 13m. Lakes Mumblin and Surprise are crater lakes. Morphometrically Mumblin is like a maar, but Surprise is elongated and complex since it occupies three contiguous craters in line, two of which are shallow, one deep.

In East Gippsland, Lake Elusive lies in a sand-dune blocked valley and is 21m deep while Lake Barracoota is a cut-off arm of Mallacoota Inlet and is 8m deep.

A bathymetric map and various morphometric parameters were presented for each lake.

SOME DIFFICULTIES IN THE DETERMINATION OF PHOTOSYNTHETIC PIGMENTS IN INLAND WATERS

by

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ABSTRACT

The available analytical procedures for the determination of photosynthetic pigments in aquatic environments totally ignore bacteriochorophyll. Necessary alterations in the procedures are to be made to account for both algal chlorophyll and bacteriochlorophyll.

INTRODUCTION

The estimation of photosynthetic pigment concentration can be based upon the optical density measured at one wave length near the red absorption maximum as described by Talling and Driver (1963), or upon measurements at several wavelengths (e.g. trichromatic method). Examples of 'trichromatic' methods include the much-used early procedures of Richards and Thompson (1952) and later modifications involving the same wavelengths of 630, 645, 665 nm as described in *Monograph on Oceanographic Methodology No.1* (1966), Strickland and Parsons (1968) and the IBP/PF Chemical Handbook edited by Golterman and Clymo (1969). All such measurements are normally corrected by subtraction of the optical density measured at 750 nm. The optical density measured at 750 nm is normally taken as an approximate measure of non-selective background absorption by other materials (also scattering and cell to cell differences). This should be normally less than 0.005 units per cm of cell thickness (Vollenweider 1969). The above mentioned procedures completely ignore bacteriochlorophyll concentrations because the optical density due to bacteriochlorophyll is not accounted for at any of the wave lengths at which optical densities are recorded. For a complete understanding of the photosynthetic activity in aquatic environment bacteriochlorophyll must also be estimated in addition to the algal chlorophyll.

All the techniques, as outlined earlier, assume the optical density measured at 750 nm to be a measure of interfering substances. In making such an assumption, errors are introduced into the techniques. They not only ignore bacteriochlorophyll concentrations, but also underestimate algal chlorophyll by subtracting the optical density at 750 nm. Bacteriochlorophyll gives a maximum peak at 772 nm, the optical density recorded at 750 nm would be due to the presence of bacteriochlorophyll (see the discussion later). When this optical density is subtracted from the optical density recorded at 665 nm, assuming it to be due to interference, it introduces a serious error by underestimating algal chlorophyll.

OBSERVATIONS AND DISCUSSION

During November 1970, water samples were collected from various depths in lakes Gnotuck and Kerlambetes in western Victoria. These were analysed for bacterio and algal chlorophylls. Bacteriochlorophyll was calculated using the tentative equation described by Takahashi and Ichimura (1968). Chlorophyll *a* was determined using the trichromatic equation described in the UNESCO Publication (1966). Bacteriochlorophyll concentrations at all depths in both localities were several times in excess of chlorophyll *a* (Hussainy in press).

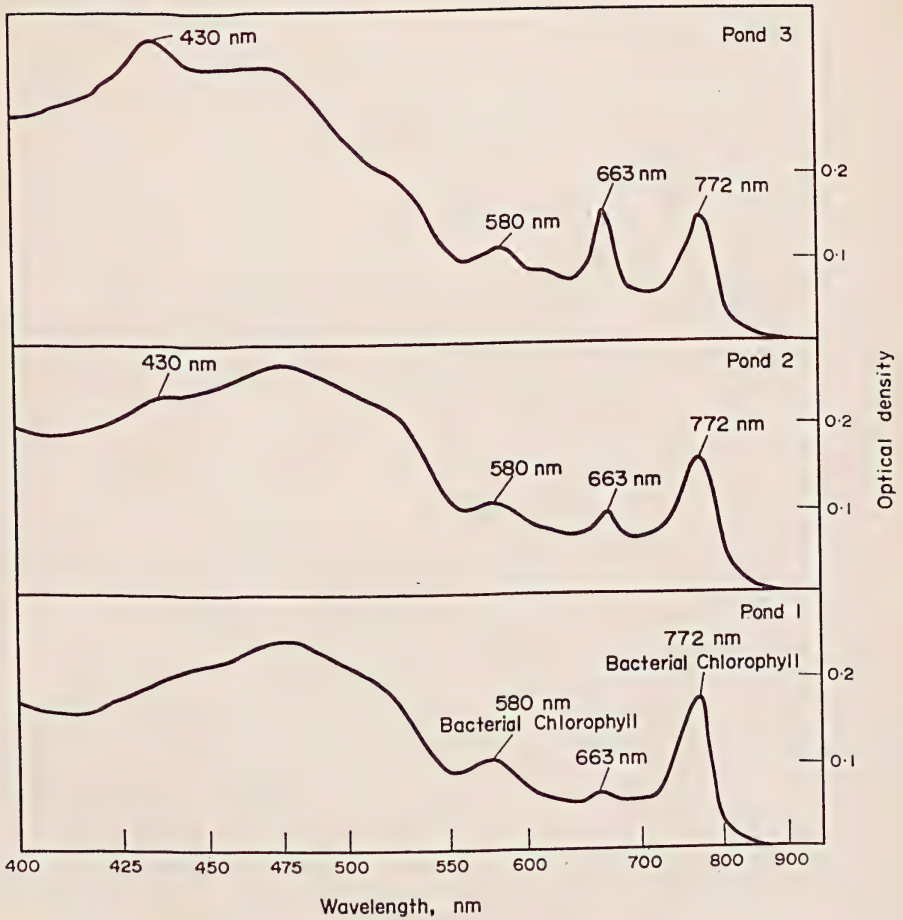

Fig. 1. Optical densities of bacterial chlorophyll and algal chlorophyll *a* in three oxidation ponds.

Another set of observations were made in a completely different type of environment, namely, the oxidation ponds used for the treatment of wastewater. The design and operation of the ponds system has been treated extensively by various authors and need not be repeated here. Purple bacteria belonging to the family Thioshodacea and Chlorobacteriacea are common in the anaerobic section of the ponds. During November, 1970, a known volume of water was filtered through membrane filters of pore size 0.45 μ. The procedure for the extraction of the pigment was according to UNESCO (1966). The extracts were centrifuged twice at 4,200 r.p.m. for 15–20 minutes on each occasion for the total removal of turbidity. A spectrograph was taken using a Beckman DK2 recording spectrophotometer. The spectrograph is presented in Fig. 1. It can be seen that for each sample there is a distinct peak at 772 nm which is either greater than or equal to the one at 663 nm. Pond 1 contained water in early stages of purification, Pond 2 was intermediate and Pond 3 contained water in the final stages of purification. All three samples show a distinct peak at 772 nm which is either greater than or equal to the one at 663 nm. With respect to the 663 nm peak, Pond 3 shows it prominently, Pond 2 moderately and in Pond 1 it is barely perceptible. Observations made on the photosynthetic pigments in these ponds since 1970 suggest that bacteriochlorophyll forms a very significant portion of the total photosynthetic pigment.

In view of the above observations, it is essential that bacteriochlorophyll be estimated in addition to chlorophyll *a* when estimating photosynthetic pigments.

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A FREEZE-DRYING TECHNIQUE FOR DETERMINATION OF TOTAL DISSOLVED SOLIDS IN BICARBONATE/CARBONATE-RICH WATERS.

by

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INTRODUCTION

The concentrations of total dissolved solids (T.D.S.) in inland waters are often estimated by determination of electrical conductivity or by evaporation of a sample to constant weight (Anon 1960; Williams, 1966). Conductivity measurements are easily and rapidly performed but are dependent on the ionic composition and level of salinity, particularly when considerable quantities of dissolved salts are present. Consequently, conductivity measurements can be converted to T.D.S. only after correlations have been established for the waters under consideration. Williams (1966) has shown a general relationship between conductivity and gravimetrically determined T.D.S. in a large number of Australian lakes over a wide salinity range and in which the ionic composition is dominated by sodium and chloride.

Problems arise in determining the T.D.S. of waters in which bicarbonate/carbonate ions constitute a substantial proportion of the anions. If the samples are dried at the standard temperature of 103°C. part of the bicarbonate/carbonate is lost due to the evolution of carbon dioxide. Drying at higher temperatures causes a further loss while at lower temperatures mechanically occluded water is retained (Anon 1960).

Because of the difficulties encountered in gravimetric determinations of T.D.S. in bicarbonate/carbonate-rich waters, studies on the applications of lyophilization or freeze-drying techniques were initiated. The results are presented as a possible aid to others interested in the limnology of such waters.

METHODS

Known concentrations of sodium bicarbonate were prepared with oven dried reagents (37°C) and distilled water. Water samples were collected from the bicarbonate/carbonate-rich lakes of the Red Rock series in Western Victoria (Bayly, 1969) by K.F. Walker and W.D. Williams from 1968 to 1970. The lakes sampled in this series have salinities that vary from about 4,500 to 50,000 p.p.m. Approximately 20 to 40% of the total T.D.S. is contributed by bicarbonate and carbonate ions. Lake samples were stored frozen in polyethylene bottles. After thawing the samples were filtered through 1.2 μ Millipore filters.

For gravimetric determination of T.D.S., 100 ml samples were dried to constant weight at 103 ± 1° C in tared beakers.

T.D.S. by lyophilization was determined with a Dynavac Model F.D.16 high vacuum freeze drier. The top half of a Virtiss 500 ml drying flask was packed with glass wool to prevent the loss of dry salts into the vacuum system. After this modification both halves

of the flask were weighed; a 100 ml sample was added to the bottom portion, and the halves were joined with a rubber gasket. The prepared flasks were pre-frozen and stored at -45°C. The pre-frozen samples were then placed on the freeze drier for 72 hours at -85°C. After drying the flasks were equilibrated to room temperature and re-weighed.

RESULTS

The results of the T.D.S. determinations by gravimetric and freeze-drying techniques are shown in Table I. When expressed as a percentage recovery of known $NaHCO_3$ concentrations, duplicate determinations by freeze drying usually did not differ by more than 2.5%. Gravimetric determination showed a maximum range of 2.3% for six replicate samples.

TABLE 1

COMPARISON OF FREEZE-DRYING AND GRAVIMETRIC TECHNIQUES FOR ESTIMATING T.D.S. OF BICARBONATE/CARBONATE-RICH WATERS. (Numbers of determinations in parentheses.)

Sample	T.D.S. g/l. Actual A	Lyophilization B	Gravimetric C	Recovery % B/A	C/A	C/B
Sodium bicarbonate	30	28.494 (2)	18.984 (3)	94.98	63.28	66.62
Sodium bicarbonate	15	14.460 (2)	9.489 (3)	96.39	63.26	65.62
Sodium bicarbonate	5	4.739 (2)	3.191 (3)	94.77	63.81	67.33
Sodium bicarbonate	1	0.975 (4)	0.659 (6)	97.53	65.88	67.59
Lake Werowrap, 27/7/1968[1]	25.139[2]	20.034 (1)	18.655 (2)	79.69	74.27	93.12
6/8/1968	–	30.030 (1)	28.188 (1)	–	–	93.87
16/8/1969	37.225[2]	36.870 (1)	35.047 (2)	99.05	94.15	95.06
28/12/1969	–	43.464 (1)	34.966 (2)	–	–	80.45
6/6/1970	–	49.609 (1)	47.878 (1)	–	–	96.51
Lake Gnalinegurk, 11/2/1970	–	44.341 (1)	42.300 (1)	–	–	95.40
Lake Purdigulac, 11/2/1970	–	11.084 (1)	9.700 (1)	–	–	87.51
Red Rock Tarn, 29/9/1968	–	11.665 (1)	10.316 (1)	–	–	88.44

[1] Date of sample collection

[2] T.D.S. by total ionic analysis (K.F. Walker, pers. com.) and assuming negligible non-ionic dissolved material present.

DISCUSSION

The use of lyophilization for T.D.S. determinations of known concentrations of $NaHCO_3$ showed the technique to be potentially superior to the standard gravimetric method. The percentage recovery of dissolved salts from natural lake waters was not as good, but was still superior to the recovery by evaporation at 103°C. With the complex ionic composition of the lake waters tested, the gravimetric technique suggested a T.D.S. between 80.5 and and 96.5% of the T.D.S. determined by freeze-drying.

It is felt that this technique holds promise as a means of estimating the T.D.S. of bicarbonate/carbonate-rich waters, particularly when the establishment of a correlation between electrical conductance and T.D.S. by total analysis is not feasible. The present freeze-drying technique could be easily refined so that more accurate results would be obtained. Sample flasks are now available that are of one piece construction and have filters to prevent the loss of dry particles. This would negate the need for packing with glass wool and smaller, one piece flasks would facilitate more accurate weighing.

Freeze-drying T.D.S. values used in this paper were determined after a 72 hr drying period. Initial experiments indicated that samples had not dried after 48 hrs, while by 120 hrs some CO_2 was lost as a result of the high vacuum and the gradual warming of the flasks to room temperature. A more precise study of effects of drying time on T.D.S. values is required.

ACKNOWLEDGEMENTS

Dr. K.F. Walker and Dr. W.D. Williams of Monash University are thanked for useful discussion. Dr. K.F. Walker is also thanked for data on the chemical composition of Lake Werowrap. This study was undertaken while the author held a post-doctoral fellowship from the National Research Council of Canada.

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A LIMNOLOGICAL SURVEY OF THE BASIN LAKES, NALANGIL, WESTERN VICTORIA, AUSTRALIA.

by

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SUMMARY

East and West Basin Lakes are small saline maars of 50‰ and 86‰ total dissolved solids respectively. East Basin (z_m = 12 m) is warm monomictic while West Basin (z_m = 13.5 m) is meromictic, and in each there is severe deoxygenation in the bottom waters.

In each lake the zooplankton contains a species of the calanoid copepod *Calamoecia* and two ostracod species, while the benthos is composed of a ceratopogonid larva, the same two ostracod species, with the addition of the oniscoid isopod *Haloniscus searlei* in East Basin and the chironomid *Tanytarsus barbitarsus* in West Basin. Benthic standing crops are low.

INTRODUCTION

The Basin Lakes lie in adjacent craters and are subjectively similar, but preliminary studies showed that they differed considerably in salinity and zooplankton. Further, in the past, one of the lakes, East Basin, was polluted by the addition of whey from a dairy factory.

With this background it was decided to survey the lakes with the aims (a) of adding information on these two lakes to the data collected by Bayly and Williams (1966) on Western District Lakes, (b) of showing the present extent, if any, of pollution in East Basin and (c) of providing comparative data for our respective researches into the ecological physiology of calanoid copepods (G.W.B.) and into the benthos of deep saline lakes (B.V.T.)

PHYSIOGRAPHY

The Basin Lakes are maars. They lie in the craters of a multiple scoria cone (Ollier and Joyce, 1964) but only partially intersect and so each is subcircular with gentle outer and steep inner water slopes – the 'Pulvermaar' subtype of Ollier (1967).

The bathymetric maps in Figure 1 and the morphometric parameters listed in Table 1 are based on outlines from a recent aerial photograph, ground measurements and many echo-sounding transects. The morphometry is typical of that for maars, except that unlike others in the area (Timms, in preparation; Walker, 1971) their bottoms are funnel-shaped rather than flat. Hence the mean depth: maximum depth ratios are lower than is normally the case for maars (Hutchinson, 1957, p. 186).

Both maars, with their high (20-40 m) and steep inner crater walls are well protected from wind. In fact West Basin is the most sheltered maar in the Western District of Victoria.

TABLE 1

SOME MORPHOMETRIC PARAMETERS FOR THE BASIN LAKES

Parameter	West Basin	East Basin
Surface area (ha)	15.8	27.4
Catchment area (ha)	21.2	46.6
Volume ($m^3 \times 10^6$)	0.915	1.244
Max. length (m)	556	756
Max. width (m)	367	472
Max. depth (m)	13.5	12
Mean depth (m)	5.8	4.5
Mean depth : Max. depth	0.430	0.375
Length of shoreline (m)	1,606	2,131
Shoreline development	1.142	1.148
Volume development	1.42	1.125
Direction of major axis	SW ⟷ NE	SE ⟷ NW

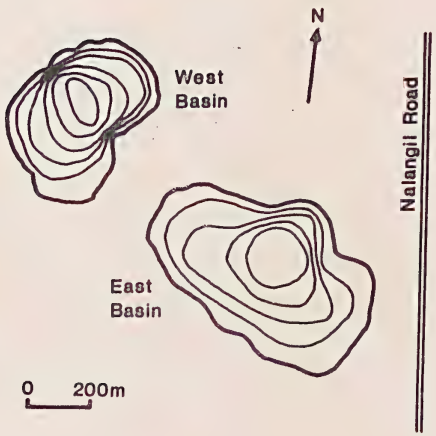

Fig. 1. MORPHOMETRIC MAP OF THE BASIN LAKES. CONTOUR INTERVALS AT 2.5 m.

PHYSICAL AND CHEMICAL FEATURES

Methods

Each lake was visited at monthly intervals for one year. Temperature was measured with an accuracy of ± 0.1°C with a resistance thermometer, dissolved oxygen by the Miller method (Walker, Williams and Hammer, 1970) and pH with a Metrohm portable pH meter. Conductivities were determined with a Phillips PR 9501 meter and converted to total dissolved solids (hereafter T.D.S.) using the formula given by Williams (1966). A Secchi Disc was used to measure light penetration and water colour was measured against methyl orange standards. Mud particle size was determined using nested graded sieves, organic matter by percent loss on ignition, nitrogen by the Kjeldahl method, and redox potential was determined using a modification of the method of Beadle (1966).

Results and Discussion

Depth profiles for temperature, dissolved oxygen and pH in mid-summer and mid-winter are given in Figure 2.

In summer both lakes stratify thermally and there is deoxygenation of the bottom layers, while in winter only East Basin circulates fully. In this lake temperature profiles for mid-October, -December, and -February indicate that stratification is of short duration – probably from November to early February. In West Basin, winter thermal stratification is associated with persistent chemical stratification (i.e. meromixis) as discussed in detail by Timms (in press).

The annual heat budget for West Basin is 7,110 g-cal cm^{-2}, while for East Basin it is 5,750 gm-cal cm^{-2}. Since heat budgets increase with mean depth (Gorham, 1964), these values are in the range expected for lakes in the area, as Lake Corangamite (z_m = 2.9 m) has an annual heat budget of 3,190 g-cal cm^{-2} and Gnotuk (z_m = 14 m) a budget of 15,477 g-cal cm^{-2} (Hussainy, 1969).

There is little light penetration in both lakes, Secchi Disc readings averaging about 80 cm in West Basin and 48 cm in East Basin. Summer readings (103 cm and 54 cm respectively) were higher than winter ones (64 cms and 44 cms respectively). Surface water colour was much the same in both – 42 Pt units in West Basin and 56 Pt units in East Basin.

Although not yet analysed, the lake water is very probably dominated by sodium and chloride ions, as is the rule in this region (Bayly and Williams, 1966; Maddocks, 1967). Both lakes are well-buffered with no discernible seasonal change in pH. In West Basin it remained near 8.4 and in East Basin near 8.6, though in the former there was a vertical decrease down to 7.0 in the monimolimnion which was probably caused by the high hydrogen sulphide content there (Timms [in press]).

T.D.S. fluctuated seasonally with a minimum in late winter and a maximum in late summer. Recorded ranges and means are 83.8-90.5‰ and 86‰ in West Basin and 48–54‰ and 50‰ in East Basin.

Mud from the deepest area in both lakes is finely divided with a large amount of organic matter and with a low redox potential (Table 2). If overseas criteria apply to Australian saline lakes then such features characterize both as highly eutrophic (Deevey,

1941; Rybak 1969). The redox potential in West Basin is one of the lowest recorded (Hutchinson 1957, p. 699), while that in East Basin is the next lowest in a series of determinations on Western District Lakes (Timms, in preparation).

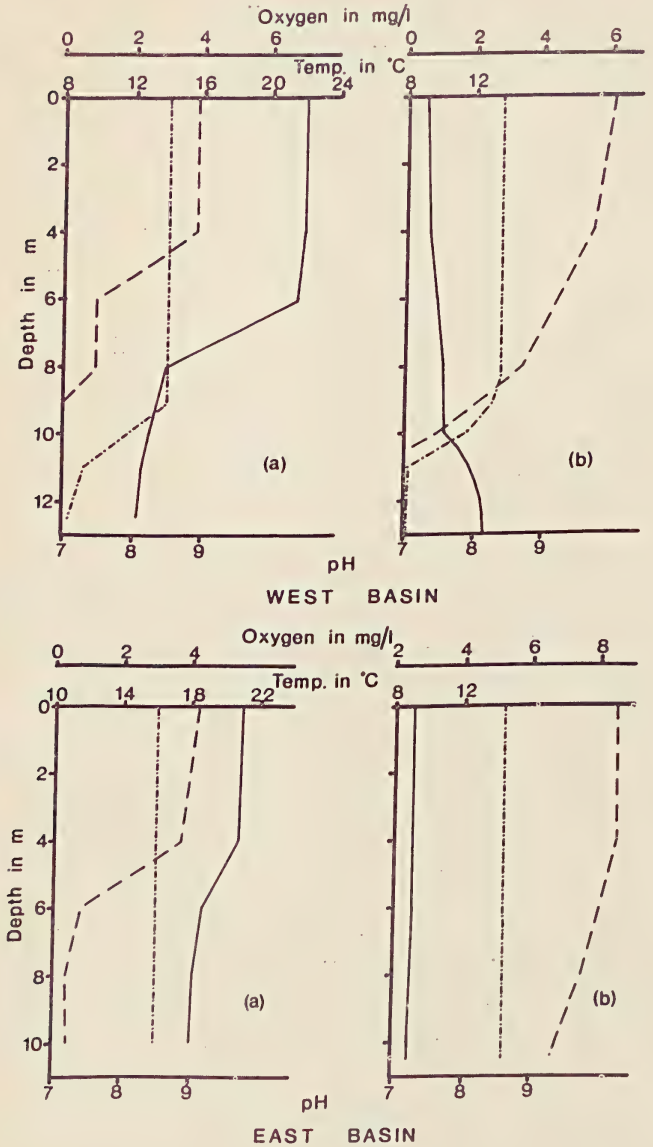

Fig. 2. Depth profiles of temperature (——), dissolved oxygen (— —) and pH (—.—.) as recorded on 17 January 1971 (a) and 19 July 1971 (b) for the Basin Lakes.

TABLE 2

SOME PHYSICAL AND CHEMICAL FEATURES OF MUD FROM THE BASIN LAKES

Feature	West Basin	East Basin
Percentage of particles <0.0625 mm	95	97
Percent organic matter	26.8	36.3
Percent nitrogen	0.82	2.47
Eh_7 in mV	−158	+109

ZOOPLANKTON

Methods

Zooplankton was collected (18.i 1971) at mid-lake stations by horizontal tows at different depths with a Clarke-Bumpus net. It was preserved in formalin. Total numbers of each species per catch were estimated by subsampling and expressed semi-quantitatively (Table 3) as numbers per propellor revolutions. (Correction was necessary as, through a technical fault, the net had to be lowered whilst open).

Results and Discussion

The zooplankton in each lake was concentrated near the surface and virtually confined to the upper few metres (Table 3). Each lake contained a single and numerically dominant calanoid copepod, the halobiont (Brand, 1972) species *Calamoecia clitellata* and *C. salina* being present in East and West Basin Lakes respectively. This appears to be the first record of *C. salina* from what is a comparatively stable salt lake (Bayly and Williams, 1966; Bayly, 1970). Both species appear to be perennial in these lakes.

The distinctness of the calanoid faunas of the two lakes warrants further study. It accords with the scarcity of recorded co-occurrences (Bayly and Williams, 1966; Hussainy, 1969), suggesting competitive exclusion. Moreover separation because of salinity difference seems unlikely (Brand, 1972), although it is not known whether production of subitaneous eggs can occur at the alternative salinities.

The ostracods *Diacypris* sp. and Cyprinae genus B *(sensu* Bayly and Williams, 1966) were present in the plankton of each lake, the former dominating the catch at 6 m in West Basin. These records of limnetic ostracods are unusual for a group whose inland-water representatives are considered benthic or littoral (but see Hutchinson, 1967, p. 695 and Bayly, 1970).

TABLE 3

ZOOPLANKTON DENSITIES AT GIVEN DEPTHS IN EAST AND WEST BASIN LAKES FOR COLLECTIONS MADE 18.i.1971. (Counts are given as numbers of individuals per counter revolution).

Depth (metres)	East Basin			West Basin		
	Calamoecia clitellata	*Diacypris* sp.	Cyprinae Gen. B	*Calamoecia salina*	*Diacypris* sp.	Cyprinae Gen. B
2	2,700	< 30	< 1	360	70	< 1
5	0	< 30	< 1			
6				130	210	< 1
8	30	< 30	< 1	12	0	< 1

BENTHOS

Methods

In mid-winter and mid-summer, triplicate samples were taken at various depths (see Table 4) with a Birge-Ekman Grab of 200 cm^2 gape, sieved through a 0.4 mm mesh, and the organisms retained sorted alive in the laboratory. Wet weights of alcohol preserved specimens were determined.

Results

In both lakes few species were present (Table 4). Numerically the ostracod Cyprinae genus B (*sensu* Bayly and Williams, 1966) and an unidentified ceratopogonid larva are the most important species. There are only two other macrobenthic species present in each lake, the isopod *Haloniscus searlei* in East Basin, larvae of the chironomid *Tanytarsus barbitarsus* in West Basin, and the ostracod *Diacypris* sp. in both. The latter is abundant but too small to be quantitatively retained by the sieve. *Haloniscus searlei* also occurs under littoral rocks in West Basin, as well as being present in the same habitat in East Basin.

There is a rapid decrease of numbers with increasing depth in both lakes (Table 4). This could result from a possible increase in the reducing nature of the sediments with depth, such an increase having been observed in Lake Gnotuk, a nearby and somewhat similar maar (Timms, in preparation), or perhaps it could be associated indirectly with poor light transmission.

The mean weighted biomasses for the two sampling dates were determined and are listed in Table 5. Cyprinae genus B contributes most to the biomass in both lakes, but the large *Haloniscus searlei* is also important in East Basin. The total standing crop in this lake is three times that in West Basin.

TABLE 4

NUMBERS OF EACH BENTHIC SPECIES (Individuals/m^2) AT DIFFERENT DEPTHS

		Cyprinae Genus B	*Haloniscus searlei*	*Tanytarsus barbitarsus*	Ceratopogonidae
West Basin Lake					
July 1970	12m	–		–	–
	8m	–		35	15
	4m	85		–	400
	1m	300		–	650
December 1970	12m	–		–	–
	8m	–		–	85
	4m	185		15	2,000
	1m	565		15	650
East Basin Lake					
July 1970	10m	15	–		15
	4m	15	35		150
	2m	3,280	515		315
December 1970	10m	–	–		515
	7m	–	–		550
	4m	15	–		700
	2m	1,785	635		985

TABLE 5

MEAN WEIGHTED BIOMASSES (g/m^2) FOR EACH LAKE

The weight at each depth was multiplied by the bottom area of the lake which that station represents. As the monimolimnion of West Basin contains no macroscopic animals, its area was not considered in the calculations.

Species \ Lake	East Basin	West Basin
Cyprinae Genus B	1.85	0.70
Haloniscus searlei	1.12	–
Tanytarsus barbitarsus	–	0.01
Ceratopogonidae	0.23	0.23
TOTAL	3.10	0.94

Discussion

The benthic species composition appears typical for Victorian saline lakes in the salinity range 40–100‰ (Bayly and Williams, 1966; Timms, in preparation). It differs considerably from that in moderately deep saline lakes in North America in which ostracods and isopods are absent and larval chironomids and ephyrids important (Rawson and Moore, 1944; Lauer, 1963).

The absence of *Tanytarsus barbitarsus* in East Basin is surprising as this species is known from salinities much lower than 48‰ (Walker, 1971) and in the laboratory it can survive in distilled water (Paterson, personal communication). Its sporadic distribution within its salinity range of 17.4–117‰ seems to be associated with an inability to compete with other chironomids (Paterson, personal communication) but this does not explain its absence from East Basin, as no other chironomid is present there.

The benthic standing crop levels observed in the Basin Lakes are in agreement with general observations of low standing crops in saline lakes and further of decreasing biomasses with increasing salinities (Rawson and Moore, 1944).

EFFECT OF INDUSTRIAL WASTES ON EAST BASIN LAKE

For about sixty years a dairy factory discharged its wastes into East Basin Lake. During the last ten years before its closure in 1960 these amounted to about 8,000 litres of whey a day and according to local opinion the lake was polluted. However, present data give little indication of this and the differences between the two lakes are largely explicable in terms of the salinity difference. Perhaps the higher organic matter and nitrogen content in the mud and the abnormally high phosphate concentration in the water (Wan, 1972) are residual effects of past pollution.

ACKNOWLEDGEMENTS

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CALAMOECIA EXPANSA (SARS) 1912: A NAME TO REPLACE THE JUNIOR HOMONYM *CALAMOECIA AUSTRALICA* (SEARLE) 1911 (COPEPODA:CALANOIDA)

by

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INTRODUCTION

In Bayly's (1961) revision of the genus *Calamoecia* the species described by Searle (1911) under the name *Brunella australis* was transferred to the genus *Calamoecia* but the specific name was not altered to agree in gender with the generic name. At the time of that revision no type or other material of an apparent senior homonym *C. australica* Sars (1908) could be located. However, subsequently (Bayly, 1962) material came to hand confirming that this senior homonym did indeed exist as a quite distinct species. In both of these papers (Bayly, 1961, 1962), therefore, the two species were nomenclaturally discriminated by the incorrect retention of the original spelling of *C. australica* and the contrasting of the two authorities "(Searle)" and "Sars". This situation should have been rectified in the second paper but by oversight was not.

PRESENT PROPOSAL

The following synonym is available to replace the junior homonym and, to rationalize the situation, it is proposed that this be adopted

CALAMOECIA EXPANSA (Sars)

Synonymy

Brunella expansa Sars, 1912, pp. 9-10, pl. viii, figs. 1-5.

Calamoecia australis (Searle). Bayly, 1961, pp. 75-8, figs. 4H, 8 A-D; 1962, pp. 254-6, fig. 2.

The diagnosis, description and figures of this species (Bayly, 1961, 1962) are, of course, in no way influenced by this nomenclatural change.

DISCUSSION

C. australica (Searle) took only page precedence over *C. viridis* (Searle) which was regarded by Bayly (1961, 1962) as the oldest synonym of this junior homonym. *C. viridis* would, therefore, in the normal course of events, have to be adopted in favour of *C. expansa.* However, as noted by Bayly (1961, p. 76) the male holotype of this species is so badly damaged and distorted that an examination of the important taxonomic features is impossible. Assignment of this nomen to the synonymy of *C. australica* was on the basis of Searle's rather inadequate description. In view of the

lack of primary type material and the brevity and shortcomings of Searle's original description, it now seems best to regard *C. viridis* as a *nomen dubium* rather than propose a neotype for it. This course opens the way for *C. expansa* which is quite adequately described and figured by Sars (1912) to be adopted in place of the rejected junior homonym.

C. expansa has so far been recorded only from Victoria and Tasmania.

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SPAWNING INDUCTION, ARTIFICIAL FERTILIZATION AND POND CULTURE OF THE MACQUARIE PERCH *(MACQUARIA AUSTRALASICA* [CUVIER, 1830])

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SUMMARY

The importance of the Macquarie perch (*Macquaria australasica*) as a food and sport fish and its apparent disappearance over some 30 years from a major segment of its former range in Victoria is discussed.

Descriptions are given of experiments on the artificial culture of Macquarie perch using fish captured from a spawning run in the upper reaches of the Goulburn River. These experiments include (1) unsuccessful attempts to induce spawning in an earthen pond and an aquarium, (2) observations of sperm activity, (3) the artificial fertilization of eggs and the examination of several fertilization techniques, (4) the incubation of eggs in glass jars, an aquarium and in a floating wooden frame, and, finally, (5) the growing of young perch in an earthen pond to which inorganic fertilizer was added.

Human chorionic gonadotrophin used at low dosage did not aid in spawning induction, nor did it induce the release of eggs from the ovaries of nearly-ripe fish. At a higher dosage rate, the gonadotrophin induced activity in a pair of fish which was probably related to spawning behaviour and the release of eggs.

Information is given on aspects of the biology of the perch including age at spawning, fecundity, egg color and size.

The paper discusses the various experiments and recommendations are made on immediate and long-term objectives for further research.

INTRODUCTION

There are perhaps six species of freshwater native fishes in Victoria which are of value from the sport-fishing viewpoint and the Macquarie perch *Macquaria australasica* (Cuvier, 1830), is one of the most important of these. It can be angled with artificial flies, the flesh has excellent eating qualities and in the early 1950's when this study commenced, fish of 1.5 kg were not uncommon at certain times and places.

The perch was once to be found generally in the cooler sections of most Victorian waters flowing into the Murray River and was abundant in the Goulburn River from its confluence with the Murray River to about Jamieson, a small village some 350 km upstream. It was also in the lower reaches of the Big, Delatite, Howqua and Jamieson Rivers, all of which used to join the Goulburn River above Eildon township and which now flow into Lake Eildon.

Lake Eildon is a hydro-electric and irrigation storage of three million four hundred thousand Ml capacity on the Goulburn River near Eildon. The dam was completed in 1956 and the lake it created inundated a smaller storage of three hundred and eighty thousand Ml which was completed in 1927.

There have been few reports of Macquarie perch in the Goulburn River below Eildon in recent years. Reputedly, they were very plentiful in this area until the mid-1930's but by 1960 they had virtually disappeared.

In the first Lake Eildon, perch were often taken by anglers but, since the lake was much enlarged in 1956, they are found only during late spring or early summer in the lower reaches of the rivers which feed the lake.

The aim of work reported in this paper was to investigate aspects of the biology of the Macquarie perch in rivers running into Lake Eildon and to attempt artificial breeding and culture of perch at the Snobs Creek Freshwater Fisheries Research Station and which is situated only a few kilometers downstream from Lake Eildon. Most of the studies were undertaken from the Station in 1962, 1963 and 1964, but some data are given in relation to Macquarie perch scales from fish captured in 1953 and 1959.

Prior to this study, few data on the species were available. McKeown (1934) and Butcher (1946) reported food items taken by Macquarie perch and Lake (1959) gave some information on spawning time, fecundity and preliminary results relating to growth rate. It was also believed that breeding had occurred in a farm dam in the north-east of Victoria (pers. comm.), but this was unsubstantiated. However there was evidence, from the ovaries of a perch which had been held in a farm pond of about one hectare surface area, quite close to the Snobs Creek Freshwater Fisheries Research Station, that eggs could develop to an advanced stage under these conditions.

Some of the terms used in the paper should be explained. In fish culture, artificial fertilization is said to be "dry" when milt and eggs are mixed together in a dry container and "wet" when water, in varying quantities, is put deliberately into the container at some stage. The basic methods and some variations are used widely and with varying success.

METHOD OF CAPTURE AND TRANSPORTATION OF FISH

The locality and the three netting sites are shown in Fig. 1.

For several weeks prior to netting operations, a watch was maintained on the lower waters of the Goulburn and Jamieson Rivers and no Macquarie perch were seen. On 20 October, a fyke net was set near the shore in the Goulburn Inlet of Lake Eildon, about 100 m from the river mouths (Site 1) in order to catch any perch that may have been moving towards the rivers for spawning. The net was cleared on 21 October and yielded 10 Macquarie perch. Nine were males and there was one female (lot 1). Milt could be taken from these nine males and all the males caught later by gently pressing the vent area. The female caught appeared to be close to spawning but no eggs could be taken by applying fairly considerable pressure near her vent area.

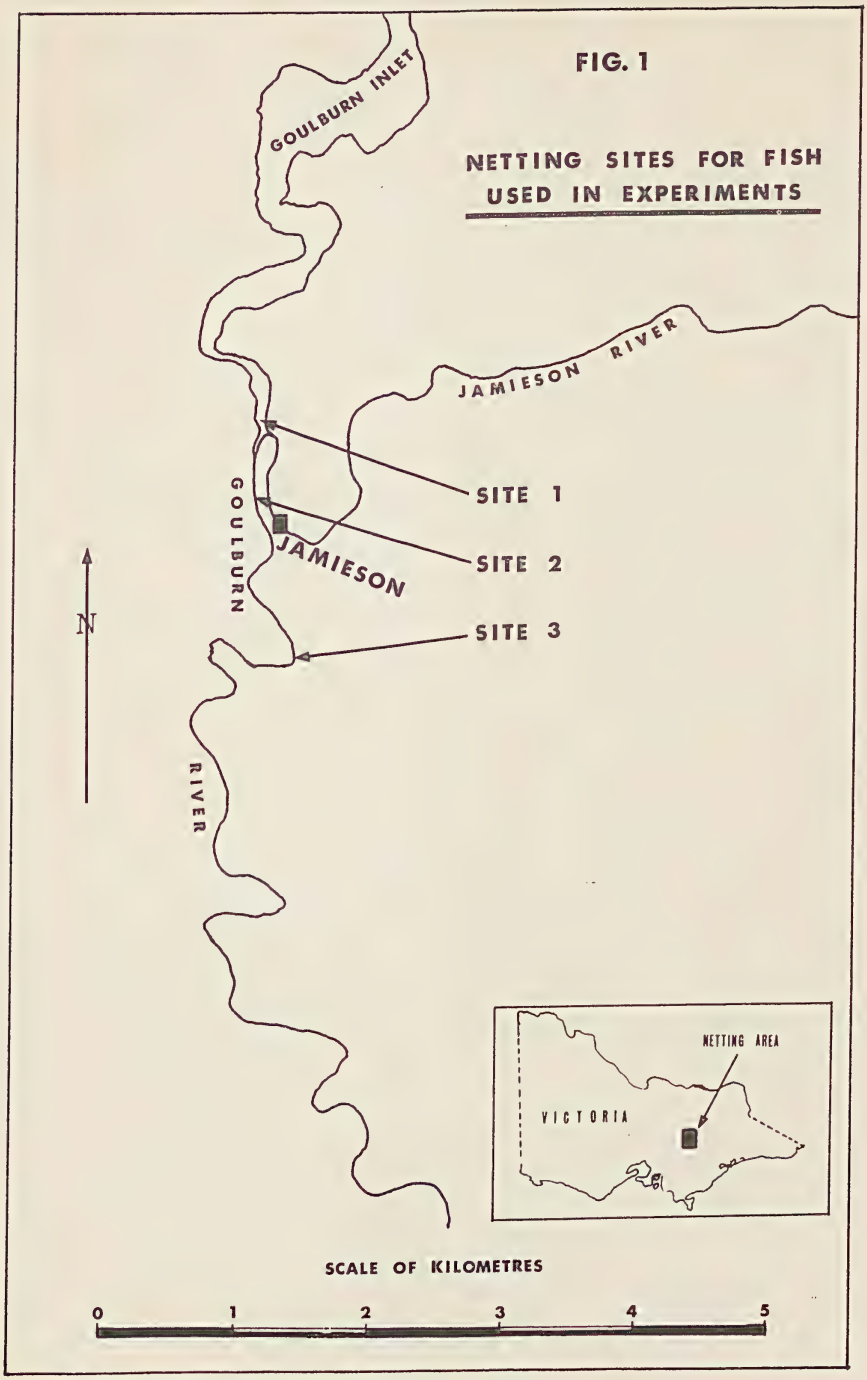
FIG. 1
NETTING SITES FOR FISH USED IN EXPERIMENTS
GOULBURN INLET
JAMIESON RIVER
SITE 1
SITE 2
SITE 3
JAMIESON
GOULBURN
RIVER
N
NETTING AREA
VICTORIA
SCALE OF KILOMETRES
0
1
2
3
4
5

On 22 October the fyke net was shifted to Site 2, about 0.5 km up the Goulburn River. It was cleared after seven days and yielded 21 fish (lot 2). Three were ripe males, five were spent females and 13 were females which were not ripe but which appeared to be close to spawning. The net was reset at Site 2 on 29 October and when cleared four days later, it yielded 19 fish (lot 3). Four were ripe males and there were 15 females which appeared to be close to spawning. The net was reset at Site 2 for the last time on 3 November and yielded 30 fish (lot 4) when it was cleared nine days later. Five fish were ripe males, 16 were spent females and nine females appeared to be close to spawning. The net was shifted and set for the last time at Site 3, which is about 1.5 km upstream from Site 2 and about the highest point at which fish were seen in the river. It was in position at about 11 am on 21 November and a large number of fish (53) were seen to enter it over a period of about 30 minutes. Four ripe males and four nearly ripe females (lot 5) were taken and of those released, 12 were ripe males, 29 were spent females and four were nearly ripe females.

Fish were transported to the Snobs Creek Station (a distance of about 48 km) on the days the net was cleared. Containers of 22 litre capacity were used and only one fish was carried in each container to eliminate the possibility of injury arising from spiking by hard fin rays. The trip took about one hour during which the fish were lightly anaesthetized with tricane methane sulfonate (MS222). On arrival at the Station, the fish were placed in large aquaria with a flow-through of water until used in experiments.

Between the 20 October and 21 November 1963, a total of 133 Macquarie perch were captured. The net was cleared on five occasions and information relating to each lot of fish, including the date and number captured, sex and gonad condition of each fish and the river temperature is shown in Table 1, together with details of fish used in experiments.

EXPERIMENTS

1. Attempts to induce spawning in a farm-type pond

The first experiment was to determine whether nearly-ripe fish captured in one of the rivers feeding Lake Eildon and placed in a specially prepared pond would spawn.

The pond used was recently constructed in heavy clay. The bottom sloped gently towards a cement-lined trench 1.0 m wide and 0.6 m deep built diagonally across the pond which facilitated the capture of fish after reducing the water level. Water entered the pond through an asbestos-cement pipe and valve built into the base of the clay bank. Water depth was controlled at a cement overflow structure built in a corner of the pond by adding or removing wooden panels. The pond water supply was from Snobs Creek which rises in similar country to the rivers which feed Lake Eildon.

The pond was filled with water on 29 May 1962 and was prepared along the lines recommended by Butcher and Thompson (1947), involving the use of inorganic fertilizers to promote plankton growth. The fertilizers were dissolved in water before being added to the pond and between 29 May and 30 September, there were nine applications. The first was a mixture of 11.36 kg of superphosphate (9.4% P), 11.36 kg of sulphate of ammonia (21.0% N), 2.27 kg of finely ground limestone ($CaCO_3$) and 2.27 kg of muriate of potash (50% K). Apart from the fifth application, which consisted of 1.36 kg of limestone, all others were double these quantities.

TABLE 1

MACQUARIE PERCH NETTED IN GOULBURN RIVER FOR EXPERIMENTS IN 1963

LOT No.	DAY OR PERIOD OF CAPTURE	RIVER TEMPERATURE (°C) *(a)*	DETAILS OF FISH CAPTURED				FISH USED IN EXPERIMENTS
			NUMBER	Sex and Condition of Gonads		WHERE CAUGHT *(d)*	
				Male *(b)*	Female *(c)*		
1	21st Oct.	14.5	10	9	1(N)	Site 1	One female killed for examination. Two males used in experiment 3.
2	22-28th Oct.	–	21	3	13(N) 5(S)	Site 2	All used in experiment 4.
3	29th Oct. to 2nd Nov.	15	19	4	15(N)	Site 2	All used in experiment 4.
4	3-13th Nov.	–	30	5	9(N) 16(S)	Site 2	14 used in experiment 1.
5	21st Nov.	16	53	16	8(N) 29(S)	Site 3	4 used in experiment 2. 4 used in experiment 4.

(a) Temperature taken on last day of capture period, usually in afternoon
(b) All males were ripe
(c) All females were either not ripe (N) or spent (S)
(d) See Fig. 1 for location of netting sites

During the experiment, water depth in the pond was maintained at 2 m and the surface area was about 0.1 ha. Daily determinations were made at about 10.00 am of minimum and maximum water temperatures, turbidity and pH. Temperatures were measured at approximately 1 m from the pond edge and 60 cm below the water surface with a maximum and minimum thermometer attached to a stake. Turbidity and pH were measured near the centre of the pond using respectively a Secchi disc and a pH colorimeter with a bromothymol blue disc and indicator. All of the data obtained, together with the dates of fertilizations, the period fish were in the pond and a short period during which a water flow through it was arranged are shown in Fig. 2.

The pond water became faintly green in colour a few days after the third application of fertilizer and this gradually became more intense over a period of five weeks. By mid-July the pond had a fairly dense phytoplankton bloom and much of the colour seemed to be readily concentrated in about half of the pond surface area by wind action. Zooplankton was also abundant and readily sampled in a plankton tow net.

On 13 November 1962, five ripe male Macquarie perch between 43.5 cm and 45 cm total length and weighing between 1.43 kg and 1.79 kg and nine perch presumed to be females (ex lot 4) between 41.0 cm and 46 cm total length and weighing between 1.35 kg and 1.89 kg were stocked in the prepared pond. All fish were marked individually by making a hole of 3 mm diameter in one or both opercula with a small punch. Four positions were designated on each operculum so that the use of one or two marks allowed for a large number of fish to be identified individually. This system proved useful but is only of value for fairly short-term experiments since the holes grew-over in about three months.

At about the same time as fish were stocked in the experimental pond, Macquarie perch eggs were found adhering to stones in clear running water in the Goulburn River (Wharton, 1968). Accordingly, a water flow-through of 15 litre per minute was arranged in the pond and this continued until 23 November. It was impractical to provide stones on the bottom of the pond.

On 14 November, a plankton net was towed repeatedly across the pond in the surface water but no eggs or fish were taken. Attempts also were made to dislodge and net any eggs adhering to the bottom of the pond by disturbing it with a metal rake and towing a plankton net so that dislodged material would be caught. These attempts were made from a small boat (see Fig. 3). No eggs or larvae were obtained. Surface and bottom netting was continued at three-day intervals until 19 December and periodically thereafter until the end of the experiment.

On 21 November a plankton net was set up in the out-flow water from the pond. This remained in position until the end of May 1963 and was inspected frequently. No eggs or larvae were obtained.

No spawning had occurred in the pond by 18 December and, since wild fish in the Goulburn River had finished spawning more than a week earlier, it was decided to treat some of the pond fish with gonadotrophin.

During late November, two dead females had been removed from the pond and one ripe male caught in the Goulburn River had been added making a total pond stock of six males and six females. On 19 December, the pond was netted and all but one female were caught. One female was killed for examination and it was noted that the

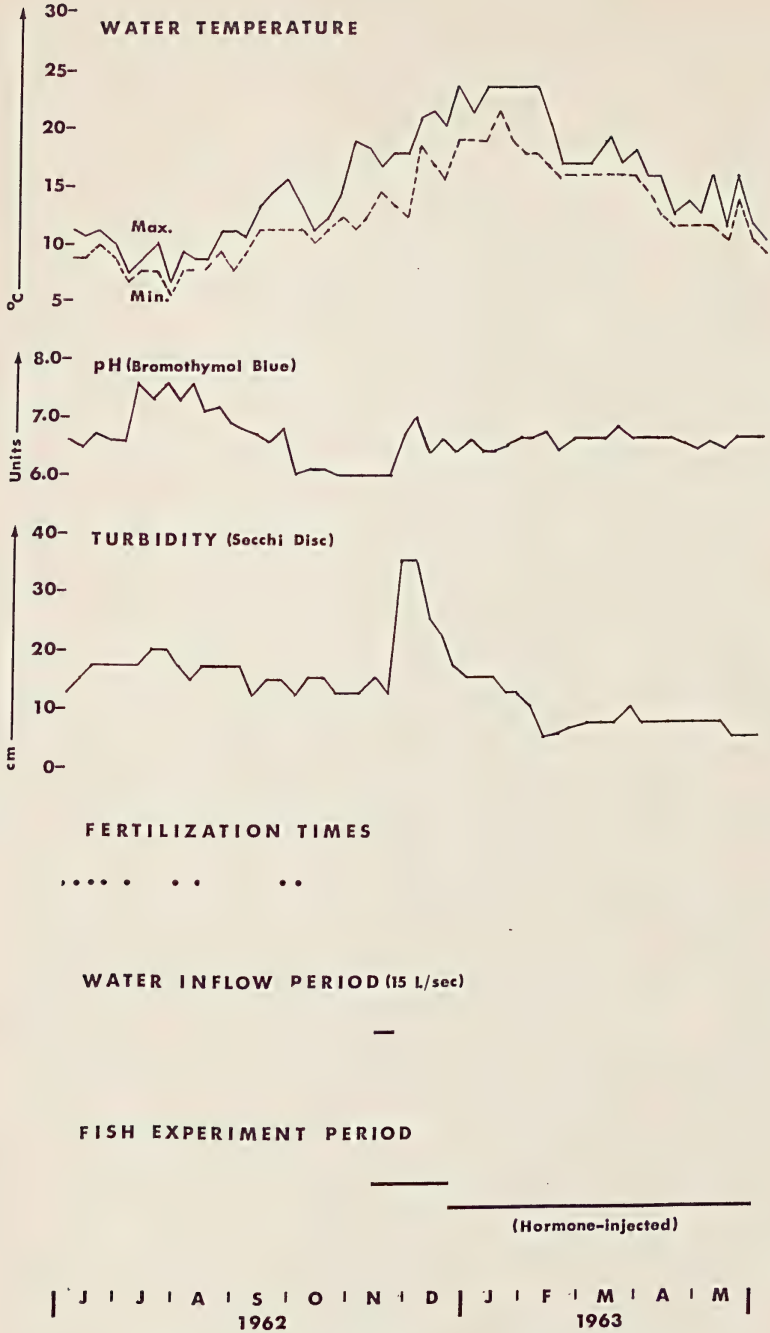

Fig. 2. Pond conditions, treatment and fish stocking period.

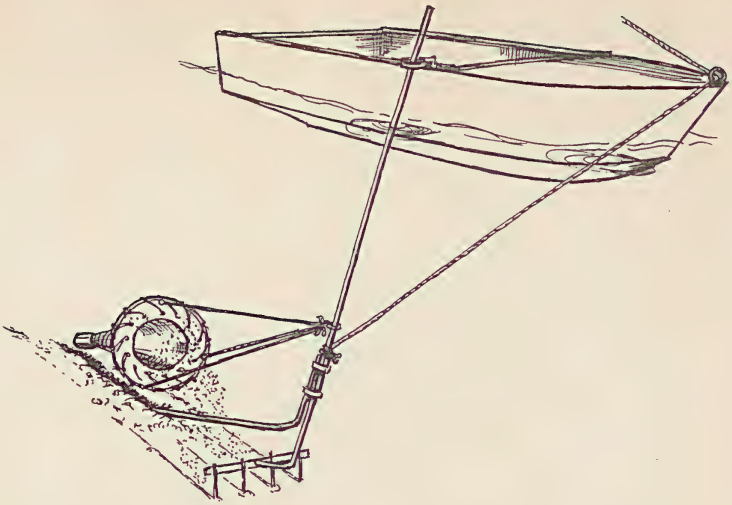

Fig. 3. Egg collecting apparatus.

eggs appeared to be resorbing in the ovaries. Two females were injected in the shoulder area with human chorionic gonadotrophin (HCG) at a dosage of 550 international units per kg body weight and were returned to the pond. The other two females were returned to the pond without receiving injections. All males received HCG injections at the same dosage as the females. Dosage of HCG was based broadly on the work of Sneed and Clemens (1959).

This experiment was concluded without positive result at the end of May 1963, when the pond was drained and eleven of the original stock of twelve perch were recovered. One HCG-injected female and one non-injected female were killed and examined. Eggs had been completely resorbed in both fish.

2. Attempts to induce spawning in aquaria

On 10 December 1962, a male and female perch caught in the Goulburn River on 21 November (ex lot 5) and held in a large indoor aquarium (4 m long, 0.7 m deep and 0.7 m wide) with a flow-through of water, were transferred to a similar aquarium which had been specially prepared as follows. Clean river sand had been placed on the bottom to a depth of 10 cm. A 7 cm thick layer of river gravel, diameter 3 – 5 cm, had been placed above the sand. There was a water flow-through of 20 litre per minute during the experiment.

The fish were allowed a few days to acclimatise and on 14 December the female, weighing 1.33 kg and of total length 42.5 cm, was injected with HCG at the rate of 1100 international units per kg (twice the rate used unsuccessfully in experiment 1). The male, weighing 1.28 kg and 40 cm long, was not injected. The following observations were made of fish in the aquarium.

17 December 1962: the female showed a noticeable swelling of the abdomen and the vent began to protrude. No eggs were seen.

18 December, 9 am: eggs were seen strewn over much of the gravel in considerable numbers. Some were floating at the surface of the aquarium and others were adhering to gravel on the bottom. Most eggs were greatly enlarged and many had burst. The female appeared deflated and both fish were removed to another aquarium. The female died a few days later.

19 December: the aquarium was cleaned and restocked with another male and female which had been caught at the same time as the first pair of experimental fish and held under similar conditions. Both fish received an injection of HCG at the rate of 1100 international units per kg. (the lengths and weights of these fish were not recorded).

24 December, 11 am: the male appeared to be actively courting the female. The male vigorously nudged the female in the vent area while she lay close to the bottom, angled to one side and with head down. The male nudged the female two to four times with his snout and then swam slowly a short distance away. He then turned on his side on the bottom, sharply flexed his body and made a small depression in the gravel. Then, returning to the female he nudged her lightly under the head and ran his slightly-open mouth along the long ray of a pelvic fin. Occasionally, he appeared to bite her quite hard on a pectoral fin and this action seemed to alarm the female who then swam some distance away. This behaviour continued, with short periods of inactivity for about one hour. In the afternoon, some clumps of eggs were seen on the gravel and these were close to small depressions made by the male.

25 December: the behaviour of the previous day was still to be observed but none of the actions were so vigorous. The female was lethargic. No more eggs were deposited in the aquarium. Most of the eggs deposited on the 24th had enlarged and burst.

26 and 27 December: no further courtship behaviour observed. The pectoral fins of the female were extensively frayed where the male had bitten them.

28 December: no courtship behaviour was observed, but about 100 new eggs were deposited on the gravel.

2 January 1963: experiment discontinued – female dead. All eggs shed in the aquarium had burst and fungus had started to grow on many.

3. Milt tests

Two of the ripe males from lot 1 were transported alive to the Snobs Creek Station for examination of milt. Urine-free milt taken from both fish in the manner described by Wharton (1957), was mixed with water in the approximate proportions of one part of milt to ten parts of water and observed under a microscope. Some sperm were active for up to one minute at 18°C. The fish were held in a large aquarium with a flow-through of water. Milt taken from both was tested daily. Sperm activation was obtained for ten days but thereafter, results of tests became inconsistent. The milt appeared to have become more viscous and did not mix readily with water. The tests were discontinued.

TABLE 2

FERTILIZATION TRIALS

Trial	Female Fish			Treatment		Eggs Taken Following Injection		Fertilization Technique(c)	Result	Comments
	Ex. Lot No.	No. Used	Holding Period (days) (a)	Eggs Taken (No.) or	Injected with (b)	Days after	No.			
A	2	5	7,2	300	–	–	–	Ed x Md	–	Eggs from spent fish
B	2	1	7,2	2000	–	–	–	Ed x Md	–	Eggs removed forcibly
C	2	1	7,2	–	HCG	8	–	–	–	No free eggs
D	2	1	7,2	–	HCG	7	(1000	Ed x Md	–	Fish seen shedding eggs
E							(1000	Ew x Md	–	
F	2	4	7,2	–	HCG	8	2000	Ed x Md	–	
G	2	1	7,2	–	HCG	8	–	–	–	No free eggs
H	2	1	7,12	250	–	–	–	Ew x Mw	–	
I	2	1	7,12	(250	–	–	–	Ew x Mw	–	
J				(250	–	–	–	Ew x Mw	–	
K	2	1	7,12	250	–	–	–	Ed x Md	+	)Development in some eggs
L	2	1	7,12	250	–	–	–	Ed x Md	+	)
M	2	1	7,12	250	–	–	–	Ed x Mw	–	
N	3	8	4,3	–	HCG	9	–	–	–	No free eggs
O	3	2	4,3	–	Dist. W.	7	300	Ed x Md	–	Fish seen shedding eggs
P	3	1	4,3	–	Dist. W.	7	35000	Ed x Mw	–	Fish weak, most eggs free
Q	3	1	4,3	–	Dist. W.	9	35000	Ed x Mw	–	Fish dead. Most eggs free
R	3	3	4,3	–	Dist. W.	10	–	–	–	No free eggs
S	5	2	. . .	5000	–	–	–	Ed x Md	+	About 90% hatch

(a) Figures on the left indicate the maximum period fish could have been in the net and on the right, the period in aquaria before trials started

(b) HCG = Human chorionic gonadotrophin; Dist. W. = distilled water

(c) Ed = dry eggs; Ew = eggs in water; Md = dry milt; Mw = milt in water

4. Artificial fertilization

In all, 19 trials were carried out, involving fish of lots 2 and 3 and several fish of lot 5. Details of all trials are given in Table 2, and before describing them individually, brief reference will be made to factors which influenced their design.

There were 15 attempts to fertilize perch eggs and eight involved dry eggs and milt (Trials A, B, D, F, K, L, O and S). In Trial L, water was added immediately after dry eggs and milt were placed together. In three trials, (M, P and Q), a water-milt mixture (sperm suspension in water) was added to dry eggs. In one trial (E), dry milt was added to eggs which had been in water for 10 seconds and in the three remaining trials (H, I and J), sperm suspensions in water were used on eggs which had been placed first in water for 1, 5 and 10 seconds respectively.

The eggs used in trials were obtained under different circumstances, as follows: found free in spent fish (Trial A); removed forcibly from the gonads of nearly ripe fish (Trial B); found free in nearly ripe fish (Trial S); found free in fish 7-9 days after injections of distilled water (Trials O, P and Q) and finally, found free in fish after a 12-day holding period in an aquarium (Trials H to M inclusive). Unfortunately, it was not possible to apply all fertilization techniques to all classes of eggs.

The hormone used in these trials and the dosage (550 international units per kg) was the same as for the pond experiment. A single injection was given to all fish.

In most trials, eggs (whether wet or dry) and milt (whether dry or mixed with water), were very gently mixed together by hand for a period of 10 seconds. After mixing, the bowl containing the eggs, milt and in some cases water, was put aside and covered for 5 minutes before the contents of the bowl were placed in water in a hatching jar. In all trials, fertilization was attempted in an enamel bowl of about 2 l capacity. About 3 ml of milt was used in each experiment and this was tested for sperm activation in water beforehand. Females were anaesthetised with MS222 and killed before eggs were dissected out and all fish were thoroughly dried with paper tissues.

Eggs from all trials were placed in urn-shaped glass jars which were 52 cm high and contained about 5 l of water which was supplied through a stiff plastic pipe running down the centre of the jar and almost touching the bottom. Water was delivered through the pipe at approximately 2.4 l per minute and this was just sufficient to keep the eggs in gentle motion in the central area of the jar and not allow them to approach the top where they could be lost in overflow water.

Individual trials will now be described:

Trial A. – Five females (ex lot 2) which appeared to be spent were killed and examined. A small number of eggs remained in each fish and these were pooled in a clean dry basin; approximately 300 eggs were thus obtained. Dry fertilization was attempted and the eggs were subsequently divided equally into two glass hatching jars. This trial was concluded on 5 November. None of the eggs showed any development.

Trial B. – A female (ex lot 2) which appeared to be nearly ripe was killed. The ovaries were dissected out, a small number of free eggs were discarded and about 2,000 eggs which were held fairly firmly in the ovary tissues were removed and

placed in a dry basin. Dry fertilization was attempted and the eggs were placed in a single hatching jar. This trial was terminated on 10 November. None of the eggs showed any development.

Trial C. A female (ex lot 2) weighing 1.5 kg and which appeared to be nearly ripe was anaesthetised and injected with HCG and then placed alone in a large aquarium. Eight days later the vent of this fish was protruding and it was netted, killed and examined. There were no eggs free in the ovaries and fertilization was not attempted.

Trial D. One female (ex lot 2) which appeared to be nearly ripe was anaesthetised, injected with HCG and placed alone in a large aquarium. After seven days the fish was seen to release a small number of eggs in the aquarium and it was caught, anaesthetised, killed and its ovaries removed. About 2,000 eggs were free and half of these were placed in a dry bowl. Dry fertilization was attempted. No egg development was noted and the trial was terminated on 10 November.

Trial E. The remainder of the eggs (about 1,000) taken from the female used in the previous experiment were placed in a bowl containing water about 2 cm deep and after the eggs had been immersed for 10 seconds, fertilization was attempted with dry milt. No egg development was noted and the trial was terminated on 10 November.

Trial F. Four females (ex lot 2) varying in weight between 1.5 and 1.7 kg were anaesthetised, injected with HCG and released into a large aquarium for observation. After eight days the vents of all fish were seen to be protruding markedly and all were removed from the aquarium, anaesthetised and killed. Ovaries were removed and a small number of eggs was found to be free in each fish. The eggs were pooled and a total of about 2,000 were placed in a dry bowl. Dry fertilization was attempted. No development was noted by 10 November and the eggs were discarded.

Trial G. One female (ex lot 2) which appeared to be nearly ripe was anaesthetised, injected with HCG and placed alone in a large aquarium. Eight days later the fish was killed for examination. The vent had not protruded markedly and there were no free eggs in the ovary.

On 10 November the remaining five females in lot 2, none of which had received hormone treatment and which had been held in large aquaria since their transfer to the Station on 29 October were used in the following trials H to M.

Trial H. – One female was killed and about 250 free eggs obtained. These were placed in a bowl containing water about 2 cm deep and about one second later, a sperm suspension made up of 3 ml of milt mixed in 100 ml of water was added to the bowl. After the usual mixing and standing period the eggs were placed in a hatching jar. There was no development visible after seven days and the trial was terminated.

Trial I. – One female was killed and about 500 free eggs were obtained. Half of the eggs were placed in a basin containing water about 2 cm deep and after 5 seconds a suspension of sperm in water was added. No development was noted in these eggs after seven days and they were discarded.

Trial J. – The remaining 250 eggs obtained from the female referred to in the previous experiment were placed in a bowl containing water about 2 cm deep and after a period of 10 seconds a suspension of sperm in water was added. The eggs were discarded after seven days as there was no development.

and after a period of 10 seconds a suspension of sperm in water was added. The eggs were discarded after seven days as there was no development.

Trial K. – One female was killed and about 250 free eggs were obtained. Dry fertilization was attempted. Examination of these eggs after seven days showed that, a few eggs had been fertilized and embryo development had begun. However, all of the eggs appeared to be dead and were discarded.

Trial L. – One female was killed and about 250 free eggs obtained. Dry milt was added to dry eggs but immediately afterwards, and before any extensive mixing took place, 100 ml of water was added and the mixture then gently swirled for a few seconds. Upon examination seven days later it was noted that some development had occurred in a small number of eggs prior to death. The eggs were discarded.

Trial M. – The last female of lot 2 was killed and about 250 free eggs were obtained. A mixture of milt and water was added to these eggs in a dry bowl. No development was noted after seven days and the eggs were discarded.

The 15 females and four ripe males comprising lot 3 were transported to Snobs Creek on 2 November and held in large aquaria until 5 November. On this date, eight females weighing between 1.58 and 2.05 kg which appeared to be nearly ripe were given hormone injections and returned to a large aquarium. The seven remaining females of this lot, which were similar in size and appearance to those which had been injected with hormone, received injections of distilled water and were placed by themselves in a large aquarium.

Trial N. – The eight hormone-injected fish were examined daily and by 14 November one fish was dead. The treatment had had no noticeable ill-effects on the others. On 15 November the seven remaining hormone-injected fish were killed and examined. There was no significant number of free eggs in any of the ovaries, either in the fish which died, or in the seven which were killed. No eggs were used experimentally.

Trial O. – On 12 November, two of the females which had been injected with distilled water were seen shedding small numbers of eggs in the aquarium. The fish were removed and killed and a total of about 300 free eggs were obtained. Dry fertilization was attempted. No egg development was noted within seven days and the eggs were discarded.

Trial P. – On 12 November, a weak female previously injected with distilled water was removed and killed. It was found that most eggs were free in the ovaries. About 35,000 were obtained and a sperm suspension in water was mixed with dry eggs. The eggs were subsequently divided into three hatching jars but were discarded after seven days. There was no sign of development.

Trial Q. – A female which had been injected with distilled water was found dead in the holding aquarium on 14 November. Dissection showed that nearly all eggs were free in the ovary. About 35,000 eggs were obtained and an attempt was made to fertilize the dry eggs with a sperm suspension in water. After mixing and standing the eggs were placed in three glass jars. No development was noted after seven days and the eggs were discarded.

Trial R. – On 15 November the remaining three females of lot 3,

all of which had received injections of distilled water ten days earlier, were removed from the aquarium, killed and examined. The number of free eggs in the ovaries of these fish was insufficient to warrant a fertilization trial.

On the 21 November, four females and four males of the 53 fish caught at netting site No. 3 (lot 5) were transported to the Snobs Creek Station. These fish were taken from the highest point in the river at which large concentrations of Macquarie perch were usually seen. The fish caught were observed to enter the net over a period of about half an hour and the time between their capture and the use of some fish in experiments was no more than six hours. Two of the females and one of the males were used in the following trial.

Trial S. – On 21 November two females from lot 5 were examined. Small numbers of eggs could be expressed from both fish. The fish were killed and all free eggs in the ovaries were dissected out. About 5,000 eggs were obtained and these were dry fertilized with milt from one of the males of lot 5. After the usual mixing and standing periods these eggs were divided evenly into three glass jars. The development and hatching of these eggs will be described in a subsequent section.

5. Fecundity, egg colour and size

Complete ovaries from three fishes used in the foregoing experiments were pooled and a number of sample counts made of weighed portions of the total egg mass. The total weight of the three fishes used was 4.89 kg and the total number of eggs in the three ovaries was calculated as 156,240. Thus, the number of eggs per kg of fish was approximately 32,000 and the number of eggs carried by a female of 1.5 kg would be about 48,000.

The colour of eggs in the ripe or nearly ripe female is a pale creamy-amber and the diameter of the unfertilized egg was 2 mm. After fertilization and contact with water the eggs become adhesive very quickly and increase in diameter to about 4 mm.

6. Age of fish

Thirteen fish were caught by handline. The first 8 were taken in April 1953 from Lake Eildon near the shoreline and about one kilometer from the point where the Delatite River enters the lake. Specimens number 9 through 13 were taken in October 1959 from the Delatite River about a half kilometer upstream from the lake in fairly slow-flowing deep water.

Scales were taken from the shoulder area of all fish, cleaned, mounted on glass plates and examined with the aid of a photographic slide projector.

The estimated ages of the fish, with lengths, weights, sexes and ovary weights are shown in Table 3.

The 1959 specimens were much larger than those taken in 1953 and their gonads all well developed although no fish of either sex were ripe.

These five larger fish all appeared to be five years old. Of the younger (1953) specimens gonads were well differentiated in two of the five one-year-old fish (both males), and in two of the three two-year-old fish (both females).

TABLE 3

Age, length, weight and sex of Macquarie perch

Fish No.	Date of Capture	Total Length (cm)	Weight (kg)	Sex (a)	Gonad Weight (kg)	Estimated Age (Years)
1	19:iv:53	19.5	0.099	M	•	1
2	19:iv:53	19.5	0.099	M	•	1
3	5:iv:53	20.0	0.114	•	•	1
4	5:iv:53	20.5	0.128	•	•	1
5	19:iv:53	22.5	0.163	•	•	1
6	5:iv:53	23.0	0.163	F	•	2
7	5:iv:53	23.5	0.185	F	•	2
8	19:iv:53	25.0	0.213	•	•	2
9	16:x:59	40.0	1.38	F	0.077	5
10	16:x:59	41.0	1.18	M	0.062	5
11	16:x:59	42.0	1.61	M	0.056	5
12	16:x:59	42.5	1.68	F	0.104	5
13	16:x:59	42.5	1.82	F	0.146	5

(a) M = male; F = Female

• = Not determined

7. Incubation of eggs; hatching and feeding of young fish

The following account is of the development of eggs used in Experiment 3, Trial S.

Daily maximum and minimum water temperatures during incubation and the early life of the young fish are shown in Fig. 4 together with information relating to the hatching period and the commencement of feeding.

Six days after fertilization, eye pigmentation was just visible in some embryos and after nine days the eyes were clearly visible in virtually all eggs. During the first week of incubation, a small number of eggs (perhaps 2%) which presumably were not fertilized, enlarged and burst, rose to the surface of the jar and were carried away in overflow water. Seven days after fertilization, the number of "eyed" eggs was estimated by hand counting a sample and relating the water displaced by the sample to the volume displaced by all of the eggs. The total number was estimated to be 4,800.

Between 29 November and 3 December, 1,280 "eyed" eggs were removed from the jars and used in experiments which will be referred to later.

On the morning of 4 December, it was found that about 1,000 eggs had been lost from one jar. A device restricting the water-flow to the jar had broken and the greatly increased flow carried eggs away in overflow water.

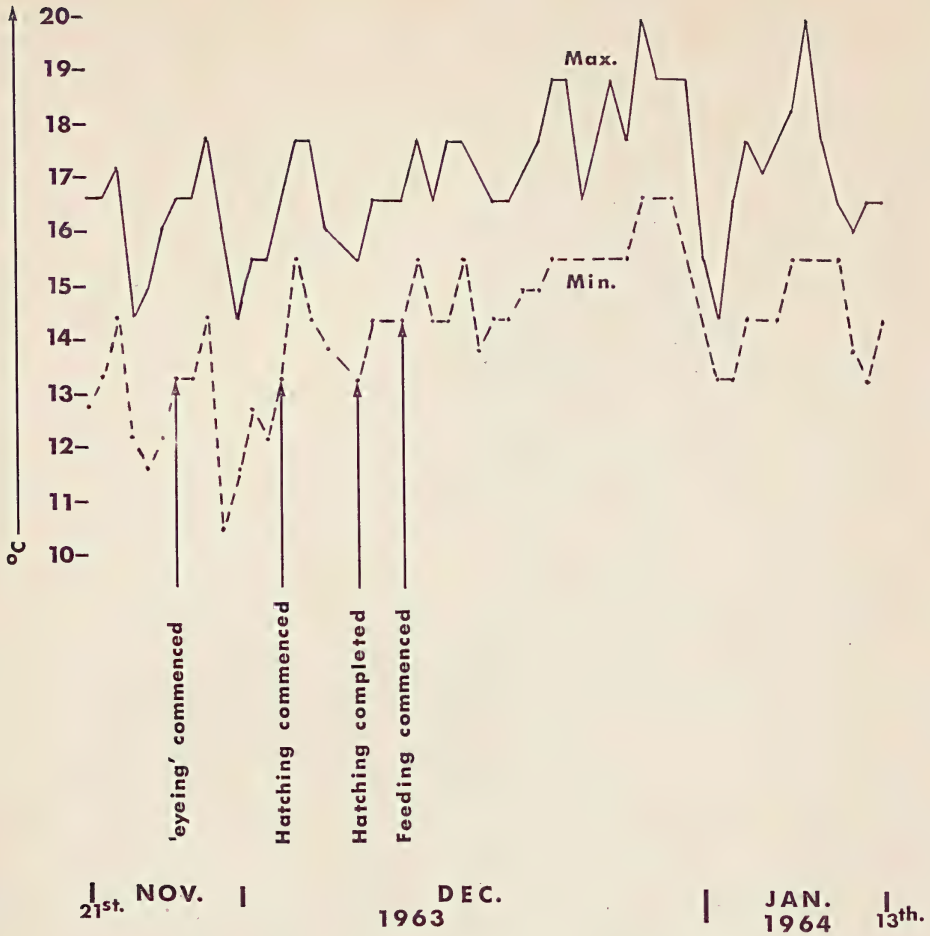

Fig. 4. Incubation of eggs, water temperatures and stages of development.

Hatching commenced on the afternoon of the 13th day after fertilization and finished on the 18th day. The day after hatching had concluded, 50 young fish were transported to an experimental pond which will be referred to later.

On 11 December, two days after hatching had finished, it was noted that the numbers of fish had decreased considerably. Overflow water from the jars was strained through plankton nets and no fish had escaped in this way. There were some fish remains in all jars and although no fish were observed attacking others, they were extremely active and fed quickly on live zooplankton, making it seem certain that cannibalism had reduced their numbers. Plankton was placed daily in all jars from 15 December, but by that time the number of fish remaining was small.

Two hundred and thirty of the "eyed" eggs removed from the jars in early December were placed in an indoor aquarium of about 15 l capacity with a small flow-through of water. One hundred of the eggs were allowed to settle to the bottom of the aquarium. A further 130 eggs were placed in a container which consisted of a 10 x 10 cm square of stainless steel wire mesh fixed to the bottom of a light wooden frame which floated in the aquarium with the wire mesh about 1.5 cm below the water surface. The mesh size of 1.6 mm was large enough (as shown later), to allow the newly-hatched fish to pass through.

More than 90 percent of the eggs on the bottom of the aquarium and in the floating tray hatched successfully and by 10 December the small fish were swimming actively in the aquarium. On 11 December, it was noted that the number of fish in the acquarium had decreased substantially. Fish remains were evident, as with the losses experienced in hatching jars and it is probable that cannibalism occurred.

Commencing on 15 December, live zooplankton were fed daily to fish in the aquarium. The experiment was terminated on 4 June 1964 when seven young perch of average length 3 cm were recovered.

8. Rearing fish in a pond

The pond used in this experiment was the same one-tenth hectare unit used in experiment 1. It had been emptied in July 1963 and was refilled to a depth of 2 m at the beginning of August. Between mid-August and mid-October the pond received six treatments with inorganic fertilizers at approximately 12-day intervals. Each treatment consisted of 22.72 kg of superphosphate, 22.72 kg of sulphate of ammonia, 4.54 kg of limestone and 4.54 kg of muriate of potash. Since its first use in experiment 1, and the commencement of this experiment, no vegetation had grown on the bottom of the pond or on its clay banks.

The first stocking of the pond was with 150 "eyed" eggs on 29 November. They were placed in a floating frame of the type used to hatch eggs in the aquarium in experiment 6. A light-proof cover was placed over the frame which was also fixed securely in one corner of the pond. On 2 December inspection showed that egg shells only were present in the frame and that all fish apparently had hatched successfully.

The second stocking was with 900 "eyed" eggs on 3 December. The eggs were placed in two covered floating frames and hatching finished on 5 December.

The pond received two further stockings and these were of small fish which were hatched in the jars. The first was 50 fish on 10 December and the second was 665 five-day-old fish on 13 December. The fish were released around the edge of the pond.

Temperature, turbidity and pH were measured in the same way as for experiment 1. Records were kept from 16 December 1963 until the end of May 1964 and are shown in Fig. 5. No food was added to the pond.

During the experiment, the pond was netted four times to examine growth, and the approximate average length of fish taken on each occasion was: 7 January, 2.5 cm; 15 February, 5 cm; 1 April, 7 cm and on the 22 May, 8 cm. Only a small

sample of fish (10 – 15) was measured on each occasion but it was noted that individuals in every catch were remarkably uniform in size.

The pond was drained and the experiment terminated on 4 June when 42 fish of average length 8.3 cm were removed. During the experiment, 24 fish were removed from the pond for various purposes so that the yield over the experimental period of about five months was 68 fish from a stocking of 1,765 eggs and fish.

Fig. 5. Pond conditions during rearing of young fish.

DISCUSSION

1. Attempts to induce spawning in a farm-type pond

The fertilizers used and their relative quantities were similar to the "Formula 2" recommendation of Butcher and Thompson (1947), but the total weights used, both for individual applications and for the entire fertilization programme were about twice the quantities they recommended per unit of pond surface area. The newness of the pond and the essentially barren nature of Snobs Creek water (Affleck, 1952) prompted this action. It was also thought that the high turbidity of pond water, apparently due entirely to suspended clay, might be reduced more rapidly by doubling the concentration of dissolved salts. Any such effect that the fertilizers may have had could have been obscured by wind action around the edges of the pond, where the water was often made very turbid. In fact, pond water became slightly more turbid after the application of fertilizers and it cleared significantly only after a water flow through the pond was provided.

In spite of the turbid conditions in the pond, a bloom of phytoplankton occurred shortly after the third application of fertilizers and it is of interest to note that the total amount of chemicals added to the pond by that time approximated, in terms of total weight per unit of surface area, the quantity recommended by Butcher and Thompson (1oc. cit.) for most Victorian conditions.

The typical pH of Snobs Creek water is between 6.6 and 6.8 (Bromothymol blue) and Goulburn River water is similar. In the pond, four applications of fertilizers produced no significant change in pH, but there was a rapid increase to 7.6 immediately after the fifth application which consisted of finely ground limestone by itself. Alkalinity remained high for about one month, but there was a subsequent gradual decrease in pH to 6.0 over approximately three months despite four further applications of fertilizers which included limestone. This suggests that much more limestone should be used on water of this nature to avoid acid conditions and that each application of fertilizers should include a higher proportion of this material.

The pH of pond water increased steadily from 6.0 to 7.0 during the first 15 days fish were present and this was due apparently to the inflow of creek water in the period. It is possible, but seems unlikely, that pH values affected fish adversely during their stay in the pond.

A water flow of 15 l per second through the pond was maintained during the first 10 days fish were present. The size of the pond and the nature of its water supply and overflow structure made it impossible to consider matching the flows of 0.4 to 1.2 m/sec. which had been measured over perch spawning areas in the Goulburn River (Wharton, 1968). The inflow did not create any appreciable movement of water in the pond and it could not be maintained for a very long period because of the danger of flushing out the dissolved plant nutrients and plankton from the pond.

Pond temperature is one of the few features of this experiment which appears to have been satisfactory. The temperature of the Goulburn River from which the fish were taken on 12 November was not recorded but it was probably a degree or two cooler than the water in the pond where the fish were released on 13 November. On that day, the minimum pond temperature was 14.5°C and the maximum 17.0°C.

Perch were seen in spawning areas of the Goulburn River when the daily minimum water temperatures were between 12.0 and 16.0°C and maximum temperatures between 16.5 and 20.0°C (Wharton, loc. cit.). Except for the last three days of November, when daily maximum water temperatures reached a peak of 18.0°C, the curves for daily maximum and minimum temperatures (see Fig. 2), do not move outside the values referred to for the Goulburn River spawning areas and for the first 14 days fish were in the pond, temperatures appear to have been ideal for spawning.

The treatment of the experimental pond fish with human chorionic gonadotrophin to induce spawning was also unsuccessful and this may have been because ovaries were resorbing at the time of treatment (as indicated by the single female examined), or to inappropriate dosage of the hormone and perhaps to other factors.

The survival of adult fish for more than five months in a small pond is of interest. Although fish may not spawn under these conditions, it would be of value for spawning induction experiments if they matured. Feeding may be a problem.

2. Attempts to induce spawning in aquaria

In this experiment, conditions were much closer to natural spawning conditions than those of experiment 1. The water was clear, there were rocks and stones on the bottom and the temperature conditions (although not recorded), were probably suitable. The only abvious factor which was not reproduced adequately was water movement. The flow of 20 l per minute through aquaria was not sufficient to produce any obvious water currents.

In the first pair of fish used only the female was injected with hormone and this appeared to induce the release of most eggs within a few days. Both male and female received hormone in the second part of the experiment and, five days later, they exhibited responsive actions which were probably similar to courtship behaviour. Some eggs were deposited but the fish were not under close observation and it is not known whether the male attempted to fertilize them.

It seems evident that none of the eggs from either fish was fertilized.

The reaction time between injection and release of eggs in this experiment was four to five days. This compares favourably with two days recorded by Sneed & Clemens (1959) for Channel catfish given single injections of the same hormone at a higher dosage. Although controls were not used in this experiment, the fact that one fish exhibited swelling of the abdomen and protrusion of the vent three days after an injection of hormone, that both fish released eggs five days or less after the injection, and that both fish of the second experiment exhibited what appeared to be courtship behaviour, seems to confirm the involvement of HCG.

The death of both females a few days after egg laying should be compared with the good survival of hormone-treated pond fish in experiment 1. It is possible to infer that the higher dosage of hormone in experiment 2 played some role.

Further work with HCG on fish held in large aquaria is warranted. Several low-dosage injections given at intervals and commencing soon after fish are captured might prove successful. Close simulation of most of the conditions associated with natural spawning should also be possible.

3. Milt tests and artificial fertilization

Only three trials (K, L and S) of the 15 in which attempts were made to fertilize eggs produced positive results. Hormone was not involved and a dry fertilization technique was used in each case.

Considering the 12 unsuccessful trials, it seems reasonable to accept that the sperm used was viable, since donor fish were not held for a long period and there were no apparent differences in the conditions of taking and using milt between successful and unsuccessful trials.

Dry fertilization was attempted in five trials (A, B, D, F & O) of the 12 that were unsuccessful and water was involved in the techniques employed for the other seven (E, H, I, J, M, P & Q). Little can be gained from considering the latter group, except to suggest that wet fertilization may have been partly or entirely responsible for the results. Injection with hormone in Trial E. distilled water in P and Q and the poor condition of fish in these last two trials also might have played a part.

Factors responsible for the failure of the five dry fertilization trials are now considered. In Trial A, the eggs may have lost viability through resorbtion while in B, they probably had not attained it. The results of the remaining three trials in this group (D, F and O) have no obvious explanation, but it could be inferred that injections of hormone (D and F) and distilled water (O) made the eggs non-viable.

Considering the three successful trials, the death of the eggs during early development in K and L is possibly related to the long period between the capture of the donor fish and the experiment (12 days), while the overall success of S is probably related to the shortness of this period (6 hours).

Hormone had no significant effect on the release of eggs at the dosage used. Disregarding trials A and B where eggs from spent and underripe fish respectively were used and fish injected with distilled water, six thousand five hundred eggs were obtained from seven untreated fish in Trials H, I, K, L, M and S. This compares with four thousand eggs from five of the 15 hormone-treated fish in Trials C, D, F, G and N.

The effect of distilled water injections on egg yield was probably not significantly different from hormone treatment. Disregarding the large number of eggs taken in Trials P and Q, which cannot be disassociated with the approaching death of the fish concerned, two of the other five fish injected with distilled water produced 300 eggs (Trials O and R).

The question whether egg yield increased while fish were held in aquaria is of interest. Ignoring eggs removed forcibly from unripe fish (Trial B), taken from spent fish (Trial A), sick fish (Trials P and Q) and assuming that injection played no significant role, there is no evidence that a holding period was of value. In fact, the best yield came from the two fish in Trial S which were used six hours after capture. The fact that fish were captive in the Goulburn River net for up to four days (Trials N through R), or seven days (Trials A through M) before their transfer to the Snobs Creek Station is of probable significance here. Some eggs could have been lost in the net.

On the results of Experiment 3, it is clear that a supply of viable milt for future artificial fertilization of eggs will present no problems. The best chance of obtaining

viable eggs would seem to be in using fish as they are netted on a spawning run. As a means of increasing the number of free eggs, it may be worthwhile treating some females several times at 24 hour intervals with HCG at 550 I.U. per kg commencing immediately fish are caught.

4. Fecundity, egg colour and size

The number of eggs found in Macquarie perch from the Goulburn River was considerably less than the number mentioned by Lake (1959), but his figure of one hundred to two hundred thousand for a 2 lb fish was a fairly rough estimate (pers. comm). and this comment applies also to his estimate of egg diameter (1 mm) and to egg colour, which he described as being white. It is also possible that these characteristics of the Macquarie perch may vary somewhat throughout its range.

5. Age of fish

The fish which were caught by hand line in the Delatite river in October 1959 were approaching ripeness as the weight of their gonads indicates and were presumably waiting to ascend the Delatite River to spawn. These fish were estimated to be five years old and they are similar in length and weight to the fish caught in the Goulburn River in 1962 and 1963. No age determinations were made of these fish but, because no substantially smaller fish were seen at any time, the question arises as to whether Macquarie perch spawn before they are five years old.

The fact that the sex of some of the one and two-year-old fish taken in 1953 could be determined suggests that a spawning at two or perhaps three years old may be possible and if so, younger fish spawn either at a different location to the older fish or did not enter the spawning groups in sufficient numbers in 1962 and 1963 to be seen or caught. The mesh of the nets used was small enough to retain two-year-old fish.

6. Incubation of eggs: hatching and feeding of young fish

The incubation of the fertilized eggs in jars presented no problems. The percentage fertilization of eggs was high and losses of eggs and young fish during development were small. To avoid the significant post-hatching losses which were probably due to cannibalism, feeding with zooplankton should commence as the first eggs hatch. From a management viewpoint it would probably be ideal to release the young fish as they hatch, into a prepared pond where the fish have space, cover (rocks and gravel) and an ample supply of zooplankton.

The use of floating frames to hatch eggs is potentially a practical proposition but further observations of this technique relating especially to possible egg predators is desirable.

7. Rearing fish in a pond

The growth rate of young perch in the experimental pond was good and the yield of about four percent fish from the original stocking holds promise. Reproduction of these results in several ponds would allow ample opportunity to study the species and 10,000 fish fed zooplankton for a few days in jars before being released into a well prepared pond might provide enough fish for small management projects.

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